Red coralline algae and climate change: growth, magnesium concentration variability and the development of a new palaeoclimate proxy

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Signature of author

January 13, 2015

Date

Statement of Contributions

Sample collection and incubation was completed by Penelope Donohue in association with Dr. Nick Kamenos and Dr. Maggie Cusack as part of a PhD thesis project. H. Stahl and N. Hicks completed DIC analysis at the Scottish Association for Marine Science. Penelope Donohue received laboratory support from M. Cusack, V. Pheonix, S. Fitzer, G. Perna, P. Chung and H. Burdett.

I was supervised by N. Kamenos through the project planning, sample processing, Raman analysis, statistics and write-up steps of the project, with additional laboratory support from P. Donohue (mesocosm sampling), J. Gilleece (sample preparation) and P. Chung (Raman Spectrometry). Dr. LeDrew provided a supervisory role at the University of Waterloo during the entirety of the project.

Chapter 5 represents an abridged version of a manuscript submitted to *Geology* in September 2014. Methodology from the manuscript has been moved to Chapter 2, and integrated with additional methodology for Chapters 3 and 4 and supplemented with statistical assumptions.

Abstract

Past ocean acidification recorded in the geological record facilitates the understanding of rates and influences of contemporary pCO2 enrichment. High resolution proxies of pCO2 and pH can be used to reconstruct components of the palaeocarbonate system. At present, most pH reconstructions are made using boron isotopes, however, there is some uncertainty associated with vital effects and isotopic fractionation. In addition to contemporary ocean acidification, marine organisms currently experience thermal stress associated with increasing atmospheric temperatures. Here we present a study of the influences of multiple stressors on the growth and structure of a marine carbonate, predicted to occur within this century, and a novel structural proxy for carbonate chemistry; Mg-O bond strength in coralline algae. Free living *Lithothamnion glaciale* algae were incubated in control (380ppm pCO2), moderate acidification (750ppm pCO2) and high acidification (1000ppm pCO2) at ambient and enhanced (+2°C) temperature conditions for 24 months. Coralline algae growth (linear extension) was highly dependent on temperature, with +2°C samples experiencing significantly reduced growth. No significant correlation was found between pCO2 and growth, indicating *L. glaciale*'s ability to acclimatize. Relative magnesium concentration and Mg-O bond strength within the high-Mg skeleton cyclically over an annual cycle. For all seasons there was a positive linear relationship between pCO2 concentration and bond strength mediated by positional disorder of the calcite lattice. Structural preservation of the carbonate chemistry system in coralline algal high Mg calcite represents an alternative approach to reconstructing marine carbonate chemistry parameters based on skeletal structure rather than chemistry.

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Introduction

The world's oceans have a reciprocal relationship with the atmospheric climate. The physical and chemical dynamics of each vary co-dependently, fundamentally influencing the state of the other (IPCC 2013; Schubert 2006). The perturbation in the chemical balance of the atmosphere, as a result of the anthropogenic release of carbon dioxide has radiating effects on the carbon flux within the ocean (IPCC 2013). An equilibrium between the partial pressure of CO_2 at the atmosphere-ocean interface influences the state heterogeneously across the globe (Sabine 2004).

Due to the accumulation of carbon dioxide in the atmosphere of polar regions, the high latitude oceans will face the first impacts of climate change, and thus local ecosystems can be viewed as the canary in a coal mine (Fabry 2009; Sabine 2004). With such ecosystems, red coralline algae (RCA) provide complex calcium carbonate frameworks that influence the flux of inorganic carbon into the surface waters (Friewald 1994; Ries 2009; Bosence 1983; Foster 2001). RCA utilize the carbon through a complex interaction of photosynthesis and calcification for growth (Bosence 1983). The ability of RCA to propagate is essential for many complex ecosystems that provide habitats for numerous invertebrates, and drive the carbon cycle in both life and decay (Nelson 2009; Wilson 2004; Freiwald 1994).

Exploring the internal structural dynamics of RCA as influenced by external environmental variables can be advantageous in investigating: 1) how carbonate organisms (and dependent ecosystems) will be influenced by climate change, and 2) how carbonate parameters co-vary abiotically and biotically to provide a depiction of climate change dynamics through paleoproxies.

The research in this thesis explores the aforementioned topics in an effort to gain an understanding of how temperature and carbon dioxide enhancements, characteristics of modern climate change, influence the canaries of the future: high latitude red coralline algae. A foundational understanding of the biology, ecology and geology of the *Lithothamnion glaciale* red coralline algae species is explored in the literature review (Chapter 1). Building upon this knowledge, the review surveys the earth system climate dynamics as they relate to the ocean carbon cycle and the intricacies of marine climate proxies.

The body of the thesis is organized in three main manuscript chapters. The leading chapter evaluates the influence of enhanced temperature and pCO_2 concentration on RCA growth. The second examines the covariance of skeletal magnesium concentration with temperature and seasonality, building upon previous paleothermometer research by Dr. Kamenos and providing a basis for the final manuscript chapter. The final chapter comprises of an abridged manuscript submitted to *Geology* in September: a novel study of the Mg-O bond strength in coralline algae as a proxy for pCO_2.

CHAPTER 1
Literature Review:
Red Coralline Algae, Ocean Carbon Cycle & Climate Change

1. Westcoast Scotland

CHAPTER 1 PURPOSE

The purpose of Chapter 1 is to provide background knowledge on the structure, growth, ecology and distribution of red coralline algae, while contextualizing their significance in palaeoclimate proxies and the oceanic carbon cycle. The current status of two research themes will be discussed:

1. Implications of ocean acidification on red coralline algae;
2. Existing marine climate proxies and their relative reliability.

This thesis aims to build on the current knowledge of the two preceding themes by analyzing the variable influence of thermal and hypercapnia stress on red coralline algae and testing a novel proxy for pCO_2.

1.1 Red Coralline Algae

1.1.1. Classification

Coralline algae are an entirely marine Order of the Phylum, Rhodophyta (red algae), categorized by their branching thallus appendages composed of calcium carbonate (Irvine 1977). These organisms have two rudimentary forms: articulated (gentricate, Figure 1.1a) and non-articulated (non-gentricate, Figure 1.1b)(Wilson 2004). Articulated forms are characteristically similar to trees, with branching thalli growing from a base of substrate grasping holdfasts. Such tree-like forms have both hard calcified and flexible uncalcified segments. In contrast, non-articulated coralline algae grow as delicate leafy or rigidly bound crust matrixes, often with protruding branches. These crusts propagate on various surfaces including rocks, algae, corals or hard shells, with both parasitic and endosymbionent roles (Bosence 1983a; Irvine 1977). The paper will focus on the articulated coralline algae form, hereafter referred to as maerl.

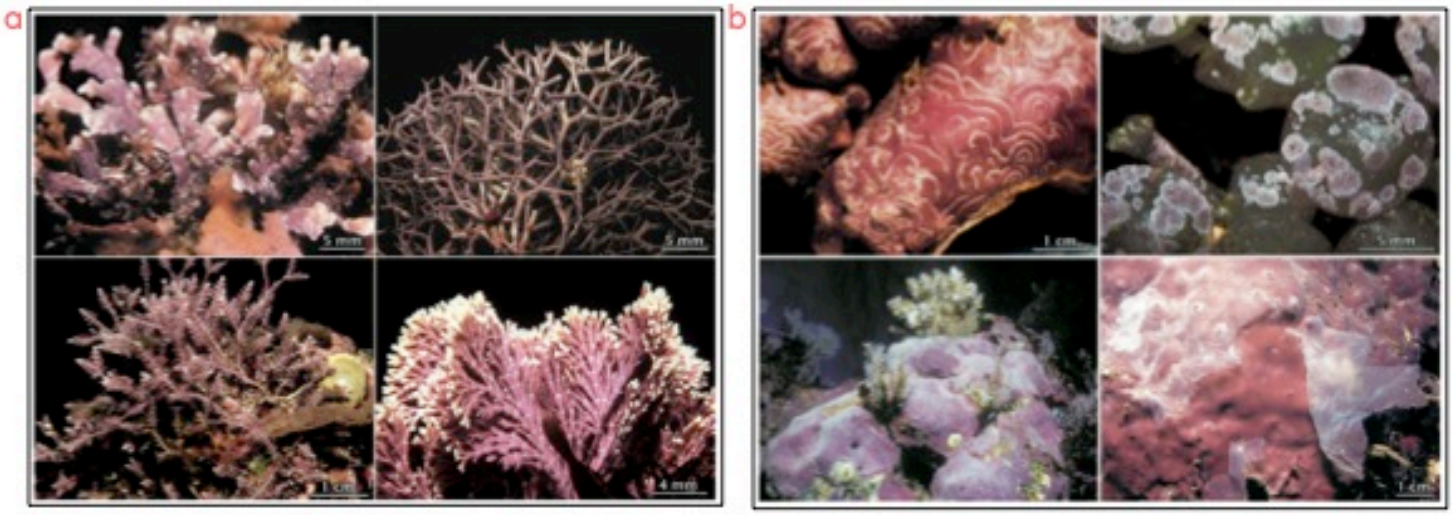


Figure 1.1: (a) gentricate and (b) non-gentricate red coralline algae forms (Littler 2013)

Maerl beds are masses of allochthonous free-living red coralline algae (Wilson 2004; Barbera 2003), accumulated by the fragmentation from autochthonous growing sites (Bosence 1983b; Birkett 1998). These maerl beds are mobile communities composed of both living and dead coralline algae fragments (Wilson 2004), of one or more species (Bosence 1983a) with or without a core of an alternative material (Freiwald 1994). Beds have been understood to collect over thousands of years, sometimes growing in excess to 2 m/ka (Halfar et al., 2001; Freiwald 1991).

These beds can develop simply from fragment accumulation or in addition to spore development (Foster 2001, Bosence 1985a,b). Maerl beds found in isolated areas typically signify initiation by solitary spore development (Foster 2001). As the maerl bed community develops, with the slow growth of live individuals, a rigid three-dimensional lattice structure forms, made up of interlocking thalli (Bosence 1983a; Birkett 1998). This lattice structure supports a high diversity of associated algae and invertebrate species, as discussed in the ecosystem section (Birkett 1998). Particular correlated epifauna grow on the thalli structures (epiphytes), providing auxiliary lattice support (Bosence 1983a, Foster 2013).

The precise lattice structure in maerl beds is a function of water energy and sedimentation (Figure 1.2).

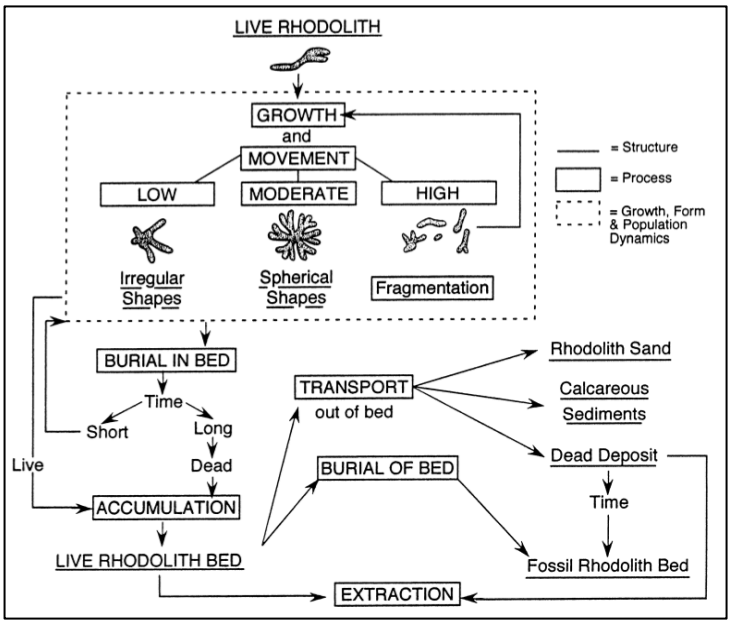


Figure 1.2: The structure of red coralline algae rhodoliths (also referred to as maerl) is a function of water movement. Rhodolith beds and associated geological features are influenced by burial, accumulation, transport and time (Foster 2001).

Weaker, delicate, open framework beds are found in low energy environments, vulnerable to fragmentation. In contrast, strong, dense framework beds are an adaptive response to moderate-energy environments (Foster 2013; Bosence 1983a). Very high-energy environments may lead to frequent fragmentation and dissolution of beds (Foster 2001). Additionally, frameworks in either environment may experience structural weakening during periods of high sedimentation, storms or low tide, preventing optimal growth (Foster 2013). For this reason, maerl beds are generally defined by the astronomical low tide to prevent desiccation (Birkett 2003). Long periods of sedimentation burial may lead to bereavement, and accumulation of deceased material, while short-term burial results in irregular thalli forms (Foster 2001). Optimal growing habitats depend on a balance of water depth and water clarity to allow ideal irradiance penetration for photosynthesis (Birkett 2003). Seasonal

variations in temperature and irradiation are important factors in high latitude maerl bed growth, as discussed in the Geography section.

1.1.2. Ecosystem Dynamics

Maerl beds have a distinguished importance within the marine biosphere, acting as ecosystem engineers (Nelson, 2009) owing to their ability to construct complex calcium carbonate frameworks (Wilson 2004; Barbera 2003). Many microhabitats exist within maerl bed frameworks amid branches, between individuals, bored into branches and buried in the underlying sediment (Freiwald 1994)(Figure 1.3). A multitude of algae, sponges, corals and other invertebrates flourish in this environmental niche at all life stages, from larval recruitment (Adey 1998), to nurseries (Kamenos 2004; Steller 2009) and ultimate settlements (Adey 1998), sometimes solitary to this particular environment (Birkett 1998). Organisms utilize the complex framework for grazing, predation and shelter (Barbera 2003). Epifaunal gastropods and crustaceans can be found living among the thalli, along with cementing bivalves and foraminifera (Bosence 1983b). Moreover, various infauna organisms live within the underlying sediment including crustaceans and bivalves (Bosence 1983b). Underlying substrates are made up of both dead remains of maerl thalli and coarse sand sediments (Barbera 2003; Freiwald 1994).

Figure 1.3: A complex rhodolith bed ecosystem with associated algae, echinoderms and gastropods; photograph from Labrador, Canada (W. Adey).

The successive manipulation of maerl beds for biological development can influence lattice structure patterns, thalli forms and density (Foster 1997; Bosence 1983a,b). Within a coral reef system, red coralline algae structures assist in framework building create hard surfaces for soft coral development (Littler 2013). In particular, coralline algae build protective boundary barriers to protect delicate coral reef systems from wave action (Dawson 1961). A delicate balance of biological interactions at varying trophic levels stabilizes the maerl bed community, precluding resource monopolization, controlling substrate solidity, regulating reproduction and controlling availability of nutrients (Barbera 2003; Grall 2003).

In addition to their biodiversity importance, maerl beds are important sources of calcium carbonate sediments in the hydrosphere and total global carbon cycle (Martin 2005; Martin 2007). Maerl fix high quantities of dissolved inorganic oceanic carbon through photosynthesis; this carbon is then precipitated in the form of high magnesium calcium carbonate within their rigid thalli structures (Bosence 1983; Foster 2001). The quantity of dissolved inorganic carbon within a specific environment can

influence organism fitness; maerl beds exposed to highly variable, stressful carbon concentrations are less sensitive to climate change associated pH deviations. However, maerl beds are generally very vulnerable to structural disintegration with ocean acidification in comparison to other algal species, because of their highly soluble magnesium calcium carbonate framework (Basso 2012).

1.1.3. Geography

Maerl beds are found on continental shelves at all latitudes, from the equator, through temperate and Arctic regions (Wilson 2004; Nelson 2009; Adey 1973) within the photic zone (Wilson 2004). Moreover, beds flourish in both open areas with strong wave action, and low-energy enclosed bays and inlets (Wilson 2004). Maerl can grow very deep around the equator, and have been discovered in excess of 300m (Littler 1991; Birkett 2003); in mid-to-high latitudes, maerl is common at depths between 1-10m (Bosence 1983b). In addition to depth differences, species composition changes with latitude (Bosence 1983a,b). *Lithothamion tophiforme* are common species in high latitude Arctic environments (Irvine 1977). L. *tophiforme* have a shiny, red-orange exterior, and evolve from nodule crusts growing on hard substrates and shells, to individually branching maerl, eventually accumulating into bed forms (Adey 2005). This species is often found in rhodolith beds along with *Lithothamion glaciale*, one of the most common subarctic and boreal coralline algae species, which has a similar growth evolution to the *L. tophiforme* species (Adey, Chamberlain & Irvine, 2005; Adey & Adey 1973) *L. glaciale* maerl have a more complex branching structure than *L. tohpiforme* (Adey, Chamberlain & Irvine, 2005), and exhibit a matte pink-purple exterior colour (Adey & Adey 1973). Further south into the temperate zone, *Phymatolithon calcareum* and *Lithothamion corallioides* species are well established into the Mediterranean, where they reach depths in excess of 60 meters (Bosence 1983b; Pérès 1967). Into the Tropics, biodiversity of maerl species increases extensively, with a dozen commonly recognized genera including *Porolithon*, *Lithophyllum* and

Mesophyllum. In comparison, temperate zones boast 5 common genera, and the Arctic only 3 (Bosence 1983b). Additionally, common species that exist at all latitudes are found at depths positively proportional to latitude (Jacquotte 1962; Bosence 1983b). This well-defined diminution of maerl diversity with latitude is a function of the severe, highly seasonal environment, as discussed in the following section.

1.1.3.1. Mid-to-High Latitudes

Maerl beds in higher latitudes are slower growing than their equatorial counterparts, but have the ability to produce equivalent quantities of carbonate (Freiwald 1994). The process of carbonate production is an important factor that permits the propagation of maerl beds in the extreme high latitude environments, as it exploits a secondary mode of growth (Friewald 1994; Foster 2001). Thalli grow to a higher degree during the summer season, when optimal light and temperature conditions are available for photosynthesis. However, in many high latitude areas, photosynthesis ceases during the winter because light is the catalyst to the reaction of converting carbon dioxide into energy (carbohydrates). In order to overcome this obstacle, maerl have the ability to (1) store summer-produced carbohydrates in starch grains for utilization in the winter (Freiwald 1994), (2) assimilate CO_2 in darkness (Kremer 1981) and (3) obtain nutrients during winter storm enrichment (resuspension of organic matter) (Luning 1990). In essence, high latitude maerl species have adapted to perform photosynthesis reactions and obtain nutrients in the absence of optimal conditions by altering life modes with annual seasonal changes. Evidence to such seasonal variations in growth and life processes is found in rhythmic, annual growth bands within the calcite thalli structure (Bosence 1983a; Freiwald 1994).

1.1.4. Growth Patterns & Seasonality

Coralline algae contain archives of previous climate conditions in a well-defined time series of biannual, seasonal growth of their distinct branch-like thallus appendages

(Adey 1970), which grow through primary production and excretion of high magnesium calcite in slow-growing bands (Adey 1970; Bosence 1983). Convex growth bands, parallel to individual thallus grow apices are visible as pairs of dark and light bands of individual cells (Figure 1.4). Each annual pair grows to a thickness of approximately 1mm in mid-to-high latitude maerl species. The dark bands, representing winter growth, are approximately half the width of the summer light bands (Freiwald 1994). Moreover, the dark bands exhibit larger, more calcified cells in comparison to the organically rich, less calcified smaller summer cells (Freidwald 1994; Bosence 1983b). The winter cells have thicker cells walls and a more circular shape due to slower cell division (Kamenos 2008; Adey 1970; Goreau 1963).

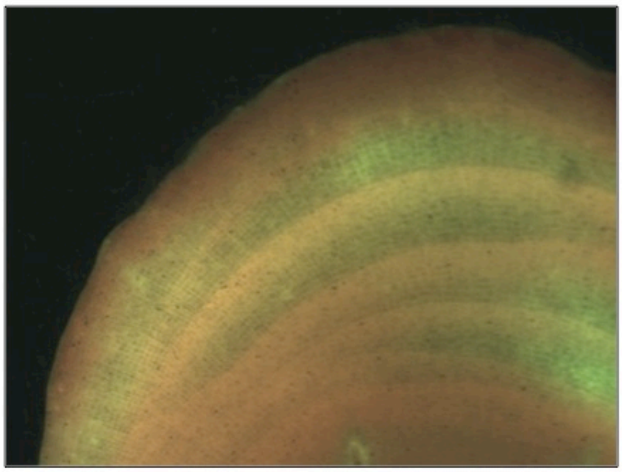


Figure 1.4: Fluorescent microscope image of red coralline algae seasonal growth bands (Maren Pauly & Nick Kamenos, 2014).

Kamenos & Law (2010) found a predictable, significant negative relationship between calcite skeletal density and temperature in the summer season; no correlation was found in the winter. This finding suggests that the growth patterns of high latitude maerl species are more regulated during optimal summer growing conditions (Kamenos 2010).

The seasonal bands exist with varying concentrations of magnesium; high magnesium concentrations correspond to enhanced growth during the spring-summer and low concentration in the reduced growth bands of the fall-winter (Heinrich 1995; Kamenos 2008). Within maerl thalli, magnesium preferentially replaces calcium within the calcite structure: $CaCO_3$ → $MgCO_3$ (Skinner 2007). Maerl have been shown to exhibit more control over the incorporation of magnesium into their skeleton during the optimal summer growth season (Bischoff 1985, Ries 2009). The incorporation of magnesium into maerl structures is a function of an abiotic replacement over calcite within the lattice at higher temperatures (Kamenos 2008; Kamenos 2009), with a proven congruent relationship (Moberly 1968; Halfar 2000).

A study by Freiwald and Heinrich (1994) found growth rate and magnitude to be inversely proportional to depth at mid-to-high latitudes, with shallower specimens growing heavier deposits at faster rates. Meanwhile, deeper specimens were discovered to have more branches per square meter, albeit relatively thinner thalli. Despite such homogeneities within depth populations, Goreau (1963) found no depth relationships, but higher growth heterogeneities within populations of different species than within the same species across vast geographical distances, indicating a strong physiological control on growth overriding localized environmental factors (Freiwald 1994).

1.1.5. Ocean Acidification

Based on their highly soluble high-magnesium calcium carbonate framework, maerl have been predicted to be particularly vulnerable to chemical changes associated to ocean acidification (IPCC 2013). Increased levels of carbon dioxide will initially enrich bicarbonate oceanic concentration, enhancing photosynthesis and calcification in marine carbonates (Ries 2009; Smith 1979). However, in accordance to the dissolution of inorganic carbon, this will lead to a decline in carbonate, which is required to form

calcium carbonate (IPCC 2013). Studies have found variable consequences to maerl, dependent on species, latitude and experimental rate of ocean acidification (Martin 2009; Ries 2009; McCoy 2014). Some studies show more resilience against decreasing pH, particularly those who naturally experience chemical variations (Noisette 2013); others describe the plasticity of maerl response dependent on the rate and magnitude of experimental conditions (Kamenos 2013; Ragazzola 2013). Other evidence suggests that secondary stressors associated with anthropogenic climate change including temperature (Chapter 3) and nutrient availability (Teichert 2014), may have more significant negative effects on maerl propagation.

1.2. Climate Dynamics, Proxies & the Ocean Carbon Cycle

1.2.1. Climate & the Earth System

Processes on earth vary through space and time as a consequence of the interactions between the biosphere, hydrosphere, atmosphere and lithosphere. These individual spheres form a network of feedbacks, which influence the daily, seasonal and annual localized weather patterns. Based on such interdependencies, weather events through time become predictably probable in different regions dependent on variation in wind patterns, precipitation, temperature, atmospheric pressure and humidity. Taken together such statistics describe a particular climate (Bradley 1999; IPCC 2013). The instrumental record is available as evidence to contemporaneous climates across the world over the past two centuries. During this time, it is well documented that anthropogenic influences, particularly the release of atmospheric carbon dioxide from fossil fuel combustion and land use change, have altered the patterns of variability in climate regimes across the globe (IPCC 2013; Alley 2003; Crowley 2000). A perturbation in one sphere has the ability to radiate through the supplementary spheres, inducing positive and negative feedbacks, which are visible within the instrumental record. A challenge arises in understanding the rates and magnitudes of present day variations and predicting future outcomes without knowledge of the

dynamics of palaeoclimatic change (Bradley 1999). This is particularly important to gain an understanding of larger scale, temporally longer mechanisms of change including continental shift, solar variability, glaciations and ocean circulation (IPCC 2013).

1.2.2. Complex Systems: Chaotic, Adaptive & Non-Linear

The Earth is analogous to a dynamic structure, as described in complex system mathematics (Bradley 1999), with characteristics of chaotic, nonlinear and adaptive systems.

In chaotic systems, the initial internal variables (in this case, Earth's spheres) may interact together in a quasi-unpredictable manner, with a multitude of possible outputs from a single input (Sterman 1994; Kellert 1994). Current knowledge of the Earth's climate system renders the variables virtually infinite. Though multiple features within the Earth system are periodic, including the Milankovitch cycles, without consistent and predictable feedback from all components, results through time are highly variable (Bradley 1999). A small time delay or variable reaction magnitude has the potential to transfer the system into a novel outcome. In time, chaotic systems experience bifurcation, the shift from inherently stable conditions, to unstable conditions and vice versa, reliant on capricious thresholds (Boccaletti 2002; Kellert 1994).

In complex adaptive systems, the subsystems and components have an inherent ability to adapt, evolve and change with internal variability (Anish 2011; Mitleton-Kelly 2003). Confined within such systems is a capacity to react based on past experience. Such a decisive reaction can materialize in a small component of the system, with radiated influences within the other components, leading to co-evolution (Kauffman 1993; Mitleton-Kelly 2003; Bradley 1999).

Inherent in both chaotic and complex adaptive systems, is nonlinearity (Sastry 1999; Kauffman 1993; Kellert 1994). This systems principle theorizes that such a system does not react according to the superposition principle: a system's net response is a simplistic summation of the reactions of its individual parts (Nelson 2008). Merely solving for a system's unknown variables does not solve its ultimate equation (Bradley 1999).

Palaeoclimate research attempts to understand previous changes in the Earth's climate by analyzing mechanisms and drivers of variability (Bradley 1999). Since the Earth system is chaotic, adaptive and non-linear, palaeoclimatology focuses on case studies of past change as each paradigm encompasses different initial conditions, internal variation, external forcing, thresholds, variable component inertias and time scales (Kellert 1994; Kauffman 1993; Bradley 1999). A diversion from average climate conditions is distinct in the palaeoclimate record as increasing/decreasing variability, upward/downward trends or spontaneous changes of a mean (Figure 1.5)(Bradley 1999). Such changes may be short-term or part of a continuous evolution into a new system state (Anish 2011). However, evidence of specific changes may be different depending on the palaeoclimate proxy utilized. Proxies from different spheres may experience global change differently in terms of rate, magnitude and characteristics (Bradley 1999; Lunt 2013; IPCC 2013).

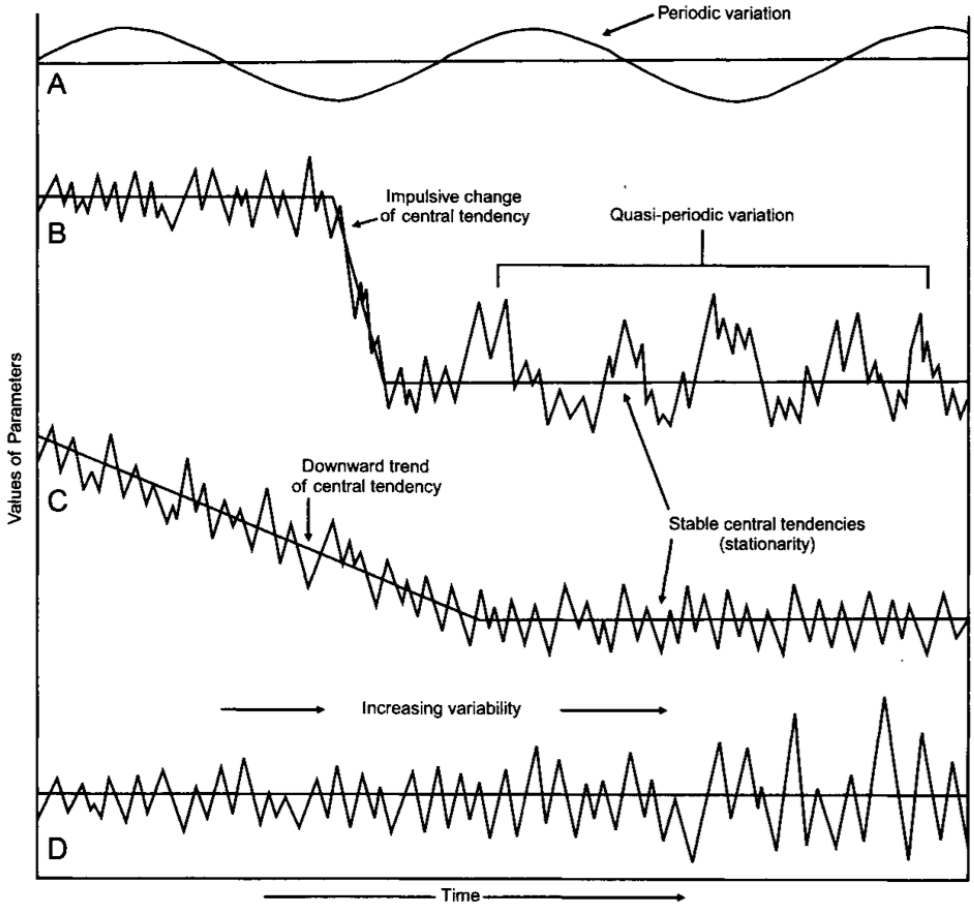


Figure 1.5: Types of climate variation and change: (a) periodic variation, (b) impulsive change and quasi-periodic variation, (c) downward trend followed by stability, and (d) increased variability (Bradley 1999).

1.2.2.1. Importance of Frequency Inconsistency

Climate change forcings may be important at different frequencies, leading to dissimilar temporal effects (Bradley 1999). For example, the Little Ice Age and "Year Without a Summer" of 1816 was a consequence of a gradual, long term forcing and an intense, short term forcing (Mann 2002; Stothers 1984). Between the 16th and 19th centuries, the Northern Hemisphere experienced colder than average temperatures, resulting in widespread expansion of glaciers and ice sheets (Fagan 2000; Mann 2002). This cooling was amplified by the sudden eruption of Mount Tambora in 1815, which

released an abundance of aerosols into the stratosphere, blocking out incoming solar radiation (Stothers 1984). In this example, the time of forcing was proportional to the resulting consequence time, however this is not always the case. In some cases, gradual, long term moderation of a climate variable may lead to a sudden change in the system because of a build-up of small component changes (Bradley 1999), as occurred during the Younger Dryas. In this instance, a large-scale melt of Lake Agassiz and resultant accumulated influx of freshwater into the North Atlantic slowed down the thermohaline circulation, eventually resulting in a geologically rapid return to glacial conditions (Teller, 2013). Moreover, apart from action-reaction related frequency discrepancies, the Earth's spheres may react at different scales to climate stimuli (Sterman 1994; Kellert 1994; Bradley 1999). For example, the atmosphere responds to chemical changes very quickly, making its composition highly variable. Since the atmosphere has a low heat capacity, it takes very little energy to change its internal temperature and chemical reactions, which require heat as a catalyst, often occur rapidly (IPCC 2013; Reay 2007). Conversely, oceans respond to external stimuli very slowly. Due to the ocean's high heat capacity, it is able to store high quantities of energy without chemical or physical modification (Calderia 2003; Reay 2007). These contradictory frequencies by which the atmosphere and ocean react to external disturbances are often clear in climate change events (Bradley 1999).

1.2.2.2. Feedback Cycles

In addition to frequency differences, climate forcings have the ability to push the system in a progressive or regressive manner, known as positive and negative feedbacks. After an initial perturbation in a system, a positive feedback arises if the supplementary reactions augment the original change and a negative feedback if they act in reverse (IPCC 2013). Within the Earth system, the growth of new vegetation in a barren region can create a positive feedback by inducing absorption of more solar radiation. This radiation is used in photosynthesis to promote vegetative growth and

evapotranspiration. Supplementary evapotranspiration increases cloud development and precipitation, providing water for additional plant growth. (Liu 2006; Woodward 1998) In the same way plants promote a positive feedback through lowering a regions' albedo, high latitude sea and land ice can induce a positive feedback through ice growth and increasing albedo (IPCC 2013). Conversely, an example of a negative feedback exists at the ocean surface. As the sea ice melts and the ocean is subjected to higher quantities of solar radiation, the surface waters warm, promoting phytoplankton growth. It is theorized that some phytoplankton species will produce more dimethyl sulfide (DMS) in these conditions, releasing it into the atmosphere (Charlson 1987). Once DMS enters the atmosphere, sulfate aerosols are produced which lead to high concentrations of cloud condensation nuclei, producing clouds and reducing the amount of solar radiation reaching the surface of the ocean (Burdett 2011). A complete understanding of such positive and negative feedbacks is vital to predicting past and future climate dynamics (Bradley 1999).

On the whole, the Earth's climate is a complex system with chaotic, adaptive and nonlinear characteristics made up of the hydrosphere, biosphere, cryosphere, lithosphere and atmosphere subsystems. Understanding how these subsystems function interdependently in the presence of internal variability and external forcings in the geological past sheds light on current processes and future projections. This paper will focus on the carbon-related anthropogenic influence on the Earth's climate system.

1.2.3. Palaeoclimate Proxies

Numerous proxies exist throughout the lithosphere, biosphere, cryosphere and ocean, which trace climatological and environmental states that existed on Earth during deposition or growth (Figure 1.6) (Cronin 2013; Bradley 1999). Pollen spores and tree rings provide understanding into general weather related conditions that enhance or

dampen growth potential (Liu 2002; Fritts 2012). Marine cores and terrestrial rock beds exhibit changes in organism ecosystems and sediment deposition, which are influenced by environmental niches, disturbance and hydrological variability (Bradley 1999; Cronin 2013). Ice cores trap ancient air within gas bubbles, delivering insight into atmospheric conditions at the time of snowfall (Dansgaard 1993). Calcifying marine organisms produce growths, whose composition and structure is directly a function of water temperature and chemistry (Grottoli 2001; McConnaughey 2008; Hemming 1992).

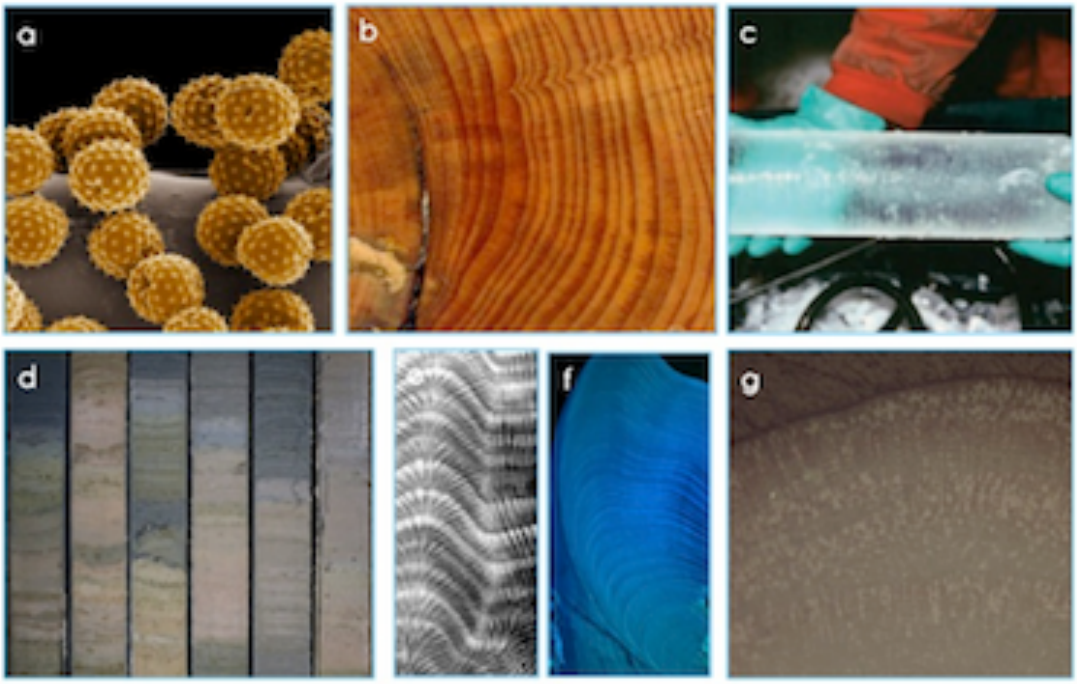


Figure 1.6: Examples of palaeoclimate proxies: (a) Pollen spores (Oak Ridge National Laboratory; NOAA), (b) Tree rings (Peter Brown), (c) Ice Core (Kennedy 2006), (d) Marine sediment cores (Hannes Grobe 2007), (e) Coral (NASA), (e) Bivalve (Alfred-Wegener-Institute), (f) Red Coralline algae (Maren Pauly & Nick Kamenos).

Each of the aforementioned proxies indicates environmental and climatological data at different frequencies, accuracy and spatial resolutions. Some proxies are more sensitive to abrupt changes and feedbacks; others lag behind change or have long

minimum sampling periods, making responses appear gradual or non-existent (Bradley 1999). Historical records, available for the past few centuries may have a variable spatial resolution, but have a low sampling interval and high quantity of climate related information (IPCC 2013). One issue that arises from historical data is the bias from the improvement of technology; early measurements were less accurate than contemporaneous ones, making estimations of patterns and rates of change difficult. Nevertheless, the sampling interval (on the day or hour scale) is unmatched by any other known proxies (Bradley 1999).

Tree rings, corals, ice and red algae are examples of proxies available at the seasonal resolution with high certainty (Fritts 2012; Grottoli 2001; Dansgaard 1993; Kamenos 2009), followed by pollen at the decade scale (Liu 2002). Speleothems, palaeosols, loess and geomorphological deposits have a resolution on the century scale, and marine deposits have a 500-year sampling interval (Bradel 1999). The temporal ranges available for these archives are generally proportional to the resolution (Bradley 1999, Cronin 2013). Individual samples of tree rings, corals and red algae have a life span of hundreds to approximately one thousand years (Cronin 2013; Frantz 2005). Continuous ice cores are generally available in the order of hundreds of thousands of years, along with pollen and speleothems. Conversely, paleosols, loess, geomorphological and marine deposits can extend into millions of years (Bradley 1999). The dynamics and disputes associated with such proxies will be discussed in subsequent sections, with particular emphasis on biological marine archives.

1.2.4. The Ocean Carbon Cycle

The carbon cycle describes the various pathways, sources and sinks of carbon in the various spheres of the Earth. Naturally, carbon is important in basic life processes of flora and fauna and by making the Earth habitable through the greenhouse effect. However, changes to this global carbon cycle could alter its vital role in the biosphere

(Sarmiento 1993 & 1998; IPCC 2013). It is estimated that 25-30% of anthropogenic carbon dioxide emitted to the atmosphere, is absorbed at the ocean surface. Within the ocean, carbon exists as dissolved inorganic carbon, dissolved organic carbon and particulate organic carbon (Reay 2007). To understanding the complex atmosphere-ocean interface and future projections of this cycle, the interactions and processes between types of carbon are important (IPCC 2013).

Each year, approximately 90Pg of carbon is exchanged in each direction between the ocean and the atmosphere. This exchange varies spatially, with some regions having a mean release and others a mean uptake of carbon (Reay 2007). The exact mechanisms behind this exchange are controlled by the partial pressure gradient between the atmosphere and the ocean (Figure 1.7, equation a) (Maier-Reimer 1987; Raey 2007). The solubility of carbon dioxide is inversely proportional to water temperature; cooler waters generally uptake more CO_2 (Sabine 2004). Once the atmospheric carbon dioxide reaches the ocean surface, it is in the form of dissolved inorganic carbon. This carbon reacts with the water to form carbonic acid (Figure 1.7, equation b), which then dissociates into bicarbonate (Figure 1.7, equation c) and eventually carbonate ions (Figure 1.7, equation d). The dissolved CO_2, bicarbonate and carbonate ions together make up the dissolved inorganic carbon in the surface ocean, with concentrations of 0.5%, 89% and 10.5% respectively (Prentice 2001). This mix of dissolved inorganic carbon drives the pH balance of the ocean (Sabine 2004; Raey 2007). Higher levels of carbon dioxide lowers pH, making the ocean more acidic; higher levels of carbonate ions increases pH, making the ocean more basic. Within this realm of the ocean, biological processes effect the carbon dioxide flux through two major processes: fixation of carbon dioxide for photosynthesis and formation of calcium carbonate shells through calcification (Prentice 2001).

The gross primary production of phytoplankton coverts the dissolved inorganic carbon into organic carbon. Approximately half of this utilized carbon is released through respiration, reproducing dissolved inorganic carbon. The remaining half remains fixed as dissolved and particulate organic carbon. At the end of the phytoplankton life cycle, this carbon either settles into the sediment, or undergoes respiration into dissolved inorganic carbon, which eventually upwells to the surface (Figure 1.7, phytoplankton cycle) (Prentice 2001; IPCC 2013). This planktonic process is known as the Organic Carbon Pump (IPCC 2013).

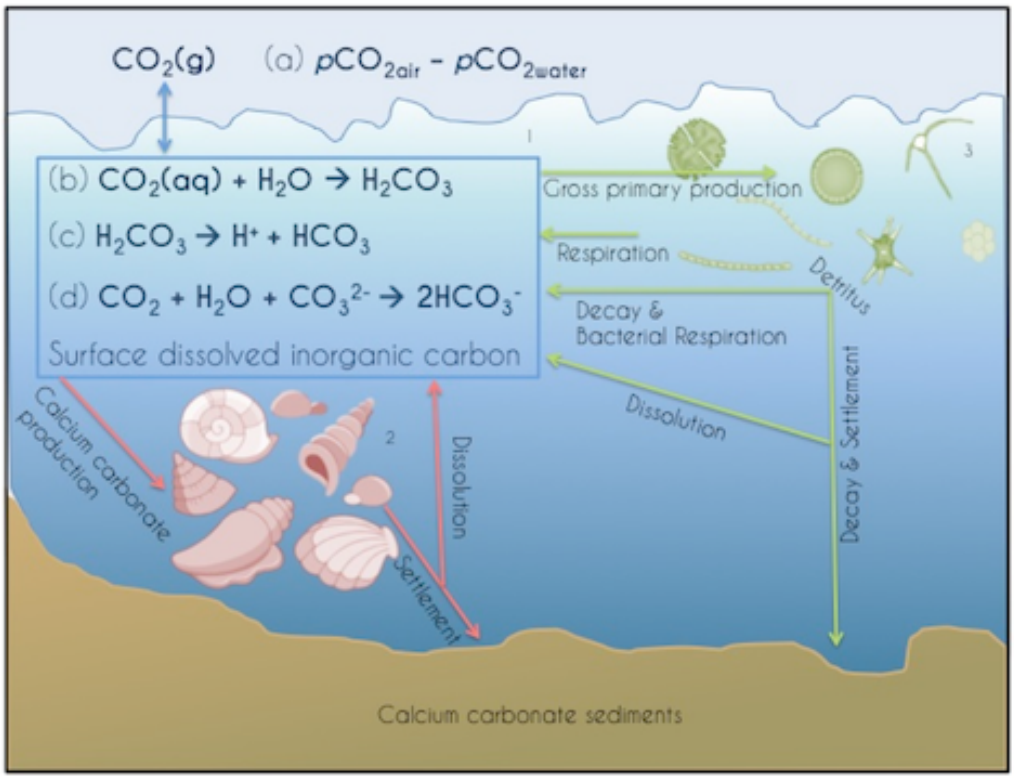


Figure 1.7: Ocean carbon cycle components, (a) pCO_2 partial pressure flux (F_{co2}) at the ocean-atmosphere interface, and (b,c,d) dissolution of dissolved inorganic carbon in the surface waters. Green arrows represent primary productivity cycling, red arrows represent carbonate organism cycling. (Created by Maren Pauly from information in IPCC 2013[1], Sabine 2004[2], Bopp & Le Quere 2009[3])

A secondary cycle, which removes dissolved inorganic carbon from the surface water involves the formation of calcium carbonate shells (Figure 1.7, CaCO3 cycle). Calcium carbonate is the basis of shells and exoskeletons of many marine organisms including molluscs, corals, red coralline algae, crustaceans and echinoderms. At the end of the calcium carbonate organism lifecycle, the exoskeletons sink to the ocean floor, contributing to the carbonate sediment load and part of the exoskeletons disperse, forming dissolved inorganic carbon. This cycle is known as the Calcium Carbonate Counter Pump (Prentice 2001; IPCC 2013).

The Solubility Pump is the main driver of the initial stage of the ocean carbon system, the temperature dependent incorporation of inorganic carbon into the surface waters (Prentice 2001). The main role of the Solubility Pump is to regulate the carbon concentration at the surface. Conversely, the principal function of the Organic Carbon Pump is to drive the movement of carbon from the surface to the deep waters. (The Organic Carbon Pump is responsible for approximately 70% of the vertical carbon movement, CaCO3 Counter Pump for 20% and Solubility Pump only 10%.) (IPCC 2013) Finally, the CaCO3 Counter Pump allows the uptake of inorganic carbon dioxide to a certain threshold, beyond which ocean acidification occurs (Raey 2007).

Ocean acidification is a natural process within the carbon cycle, with notable global events correlating to mass extinction, including the mid-Pleistocene transition (Hönisch 2012; IPCC 2013)). As carbon dioxide levels exceed the buffering capacity of the CaCO3 Counter Pump, the process of converting carbonic acid to bicarbonate and hydrogen ions reduces the carbonate saturation state and pH levels beyond biological tolerance (Sabine 2004; Doney 2009; Feely 2009). As the carbonate ions are dissolved, less is available for the growth and propagation of calcifying marine organisms (Feely 2009; IPCC 2013). This anthropogenic interference with the carbon cycle has already

negatively impacted the marine biosphere with evidence appearing in marine proxies including corals, molluscs, foraminifera and red algae (IPCC 2013; Feely 2009).

1.2.4.1. Role of the Ocean & Latitudinal Variability

As discussed in the previous section, cooler, fresh waters have the ability to absorb more atmospheric carbon dioxide than warmer, older water (Sabine 2004). Figure 1.8 depicts the spatial variability of pH levels within the oceans. Equatorial regions generally have lower pH values as they are highly saturated in CO_2. However, the thermohaline circulation drives the movement of these saturated warm waters to the regions of less-saturated cool waters, influencing local carbon inventories and biological niches (Sabine 2004; Raey 2007; IPCC 2013).

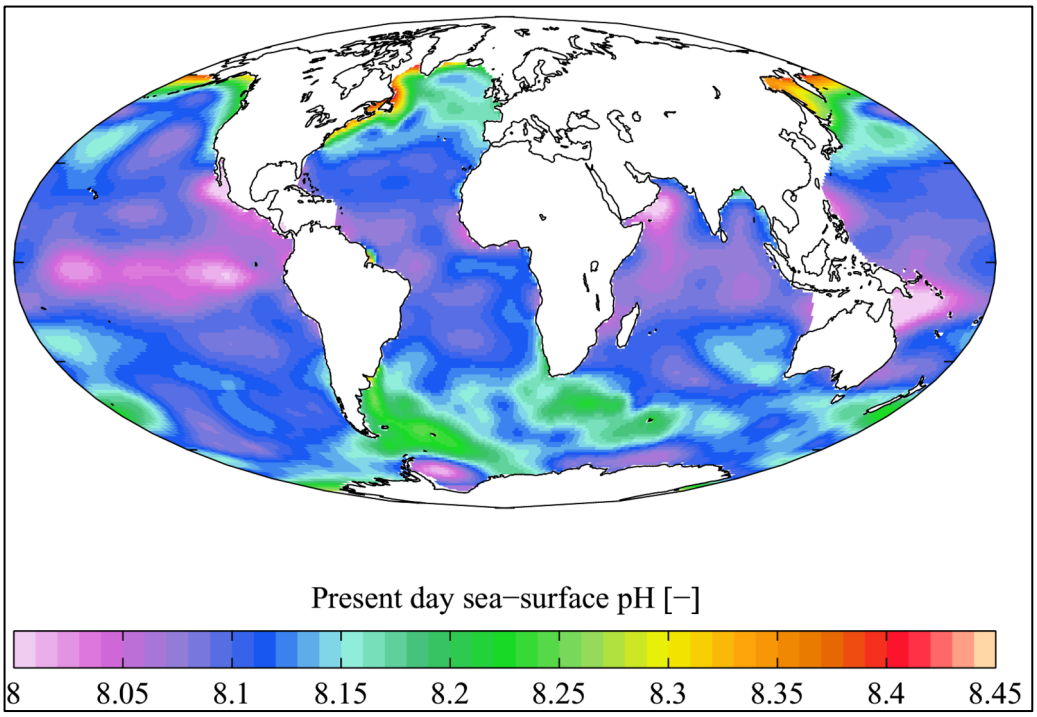


Figure 1.8: Map of estimated current surface pH levels (NASA SeaWiFS Satellite).

The North Atlantic Ocean stores almost ¼ of the ocean's total carbon dioxide (Figure 1.9) due to slow process of moving surface waters to the deep ocean, a rate-limiting stage in the global process (Sabine 2004). This thermohaline circulation influence on

the carbon storage impacts the primary production regimes, initiating the operation of the biological modes, named according to the available nutrients: Carbonate Ocean and Silicate Ocean (DeMaster 1981; Brzezinski 1985; Dugdale 1995; Sabine 2004; Edmund 1974).

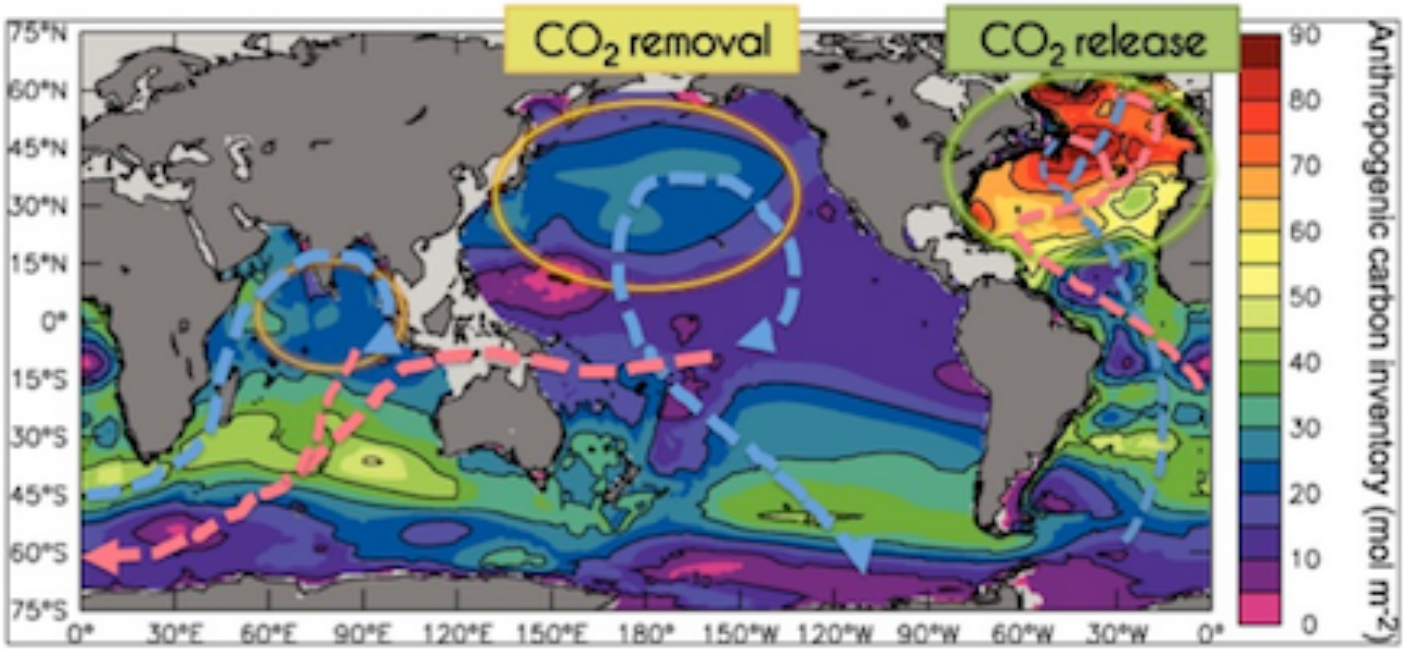


Figure 1.9: Map of anthropogenic carbon dioxide flux (IPCC 2013). Additions by Maren Pauly: red arrows indicate movement of warm water; blue arrows indicate cold water of the thermohaline circulation. Yellow circles show examples of the Silica Ocean mode and green circle shows the Carbonate Ocean mode.

The majority of the ocean area is dominated by the Carbonate Ocean mode (80%), with the remaining made up of the Silicate Ocean mode (20%), in the North Pacific Ocean and Arabian Sea (Edmund 1974; Honjo 2013). The North Atlantic Ocean is a strong example of the Carbonate Ocean mode. The warm water transferred from equatorial regions towards Greenland is high in inorganic carbon and low in nutrients (Sabine 2004). This excess of carbon is utilized by coccolithophore phytoplankton, whose high abundance is a function of the optimal chemical conditions (Honjo 2013).

Conversely, the upwelling of cold nutrient rich waters drives the Silicate Ocean mode, plentiful in silica and relatively depleted in carbonates (Sabine 2004). Diatom phytoplanktons thrive in these conditions as they utilize the hydrated SiO_2 for cell wall formation. Though the coccolithophores utilize the dissolved inorganic carbon, the Carbonate Ocean mode is a net source of carbon dioxide, and the Silicate Ocean mode is a net sink (Honjo 2013). As a consequence of the thermohaline circulation and slow layer mixing, the majority of stored carbon in the ocean exists in the surface waters (Sabine 2004). The high quantities of anthropogenic carbon dioxide being absorbed and transferred to the causes this region to be highly sensitive to the first signs of climate change as the efficiency of carbon uptake is approaching its buffering threshold.

1.2.5. Marine Palaeoclimate Proxies

Biological sources of marine proxies exist in as micro-proxies (foraminifera) and macro-proxies (corals, molluscs and red algae). Each micro-proxy has the ability to contain instantaneous environmental and climatological data from the time of shell growth (Bradley 1999). A collection of micro-proxies, as in a sediment core, is required to interpret change over time (Katz 2010). Conversely, many marine macro-proxies build layers within their carbonate structures leaving behind environmental signatures through their life span, allowing retrospective analysis (Kamenos 2010).

1.2.5.1. Corals

Large species of corals, often of the Porites genus are utilized for palaeoclimate proxy analysis based on their fast growing, annual banding, long lifespans and abundance across latitudes (Pelejero 2005; Lough 2004) (Figure 1.10). Corals have provided the basis to one of the most biodiverse ecosystems throughout Earth's history; coral reefs,

which are commonly found in equatorial regions (Veron 1995). Though coral reefs have the ability to grow at all latitudes, they particularly flourish in that environment.

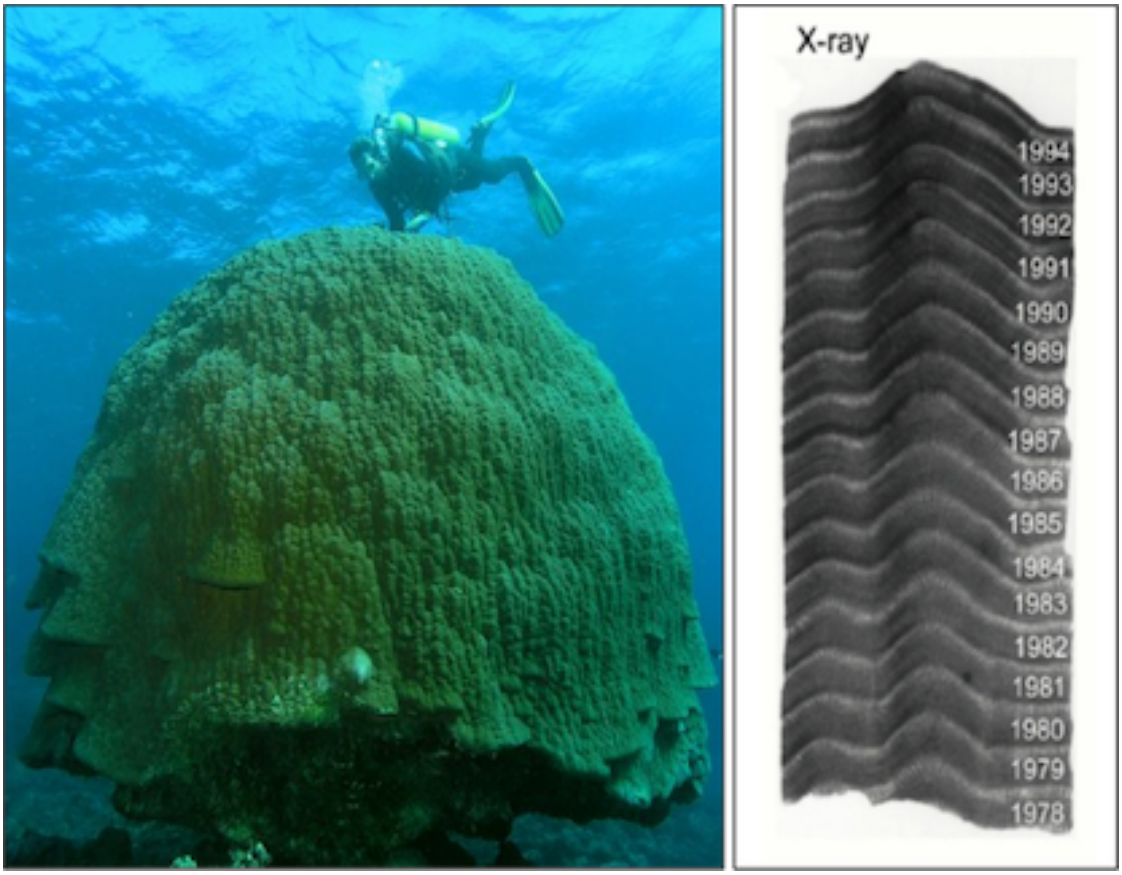


Figure 1.10: Porites coral individual (left, Eric Matson) and X-ray image of coral growth bands (right, University of Southampton).

Porites corals are part of the order Scleractinia, and form calcified hard parts in the form of aragonite, a polymorph of calcite (Veron 2000; Skinner 2003). Corals obtain required nutrients for growth primarily through both photosynthesis but also ingestion of zooplankton. Round external structures, known as polyps function as mouth appendages, filter feeding particulate matter. Livings symbiotically on the polyps are zooanthallae (algae), who perform photosynthesis (Grotolli 2001; Barnes 1968). Each year, the characteristics of growth vary depending on the season. During the optimal conditions, thick, dense layers of aragonite form, under the filter feeding

polyps (Barnes 1968). In the other months, these layers are thinner and less dense. The season-band correlation diverges depending on species and latitude, with a more pronounced band variance in regions with high seasonality (Grotolli 2001). In order to interpret palaeoclimate proxies, large individuals are cored, sliced vertically along the growth axis, and x-rayed for identification of seasonal layers. Once seasonal layers are identified, regions are micro-drilled at millimeter intervals at 30-60 day resolution (Grotolli 2001; Bradley 1999). Oxygen isotopes (δ18O) provides information on sea surface temperature and salinity and carbon isotopes (δ13C) on light and nutrients (Bradley 1999). The use of oxygen isotopes is directly related to the collection site since δ18O is higher in seawater relative to precipitation (Epstein 1953). If the site is highly influenced by variable precipitation, the δ18O only accurately depicts salinity, rather than temperature and vise versa (Grotolli 2001).

Strontium, magnesium, barium and uranium to calcium ratios (Sr/Ca, Mg/Ca, Ba/Ca, U/Ca) interpret sea surface temperature and upwelling can be estimated by cadium and barium to calcium ratios (Cd/Ca, Ba/Ca) (Grotolli 2001; Cohen 2001; Druffel 1997). Boron (δ11B) ratios can be used to approximate pH levels and qualitative skeletal growth bands can be used to evaluate growing conditions (seasons, disturbance, temperature, light) (Bradley 1999; Grotolli 2001; Trotter 2001). The complex relationship between the animalia coral and eukaryotic zooanthallae create uncertainty in the process of isotopic and trace element incorporation into the coral skeleton due to vital effects and the variable responses of photosynthesis and filter feeding (Grotolli 2001).

1.2.5.2. Bivalves

Bivalves are a group of shelled organisms in the Mollusca phylum, composed of a segmented, two-part hinged shell. The bilaterally symmetrical segments are composed of calcium carbonate, often in the form of aragonite (Barnes 1968; Dame 2011) (Figure

1.11). Bivalves have been found across latitudes and time, in both marine and freshwater systems (Dame 2011). Similarly to corals, bivalves secrete calcium carbonate in rhythm with seasonal and environmental parameters. Thick growth increments represent optimal conditions, which are separated by thin lines that represent lessened growth. The delineation of these increments are not simply temporally constrained by season; the growth pairs could represent an annual, lunar, daily or monthly cycle (Jones 1983).

In addition to varying temporal resolution of bivalve growth layers, heterogeneities exist in the geochemistry within growths deposited at the same time (Hahn 2014). Calcium paired isotopic analysis (Sr/Ca, Mg/Ca, Ba/Ca, U/Ca) have revealed variable isotopic signatures dependent on the specific calcium carbonate crystal analyzed (McConnaughey 2008; Jones 1983; Bradley 1999). This means that slight deviations in the sample pathway could produce erroneous environmental signatures. Moreover, calcium isotopes are often highly out of phase with the ambient seawater. Based on such evidence, it is theorized that circulation and nutrients are the main causes of variable trace element incorporation, rather than light, salinity and temperature (Jones 1983). Carbon isotopes ($\delta^{13}C$) have the potential to be used to qualitatively estimate river inputs and upwelling and oxygen isotopes ($\delta^{18}O$) for temperature, if ambient salinity is known (Schöne 2004; Bradley 1999). However, vital effects and ontogenic growth characteristics are thought to be foremost controls on the high uncertainty of bivalves as palaeoclimate proxies (Lorraine 2005).

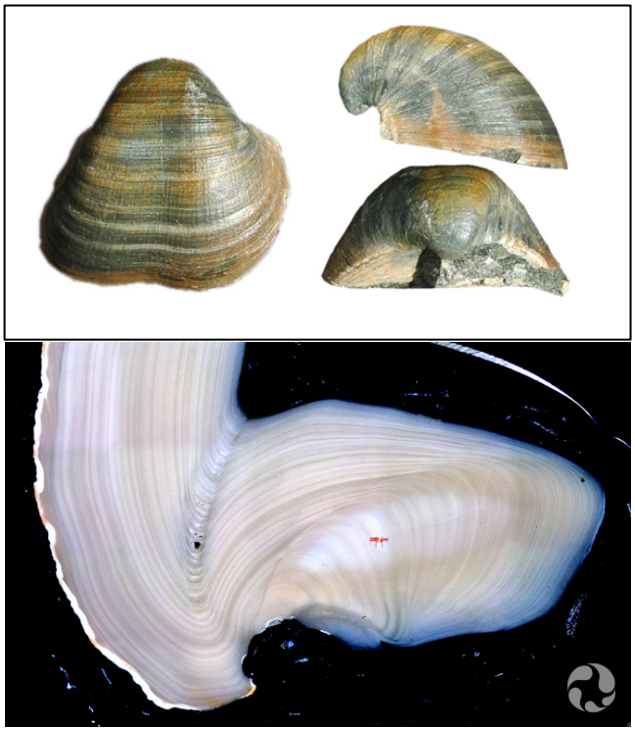


Figure 1.11: Three views of a bivalve from Vancouver Island (top), and a cross-section displaying growth rings (bottom) (Daniel Munro).

1.2.5.3. Foraminifera

Foraminifera are microscopic planktonic and benthic organisms, which commonly produce a calcium carbonate shell (coccolithopores), less commonly, a silica shell (diatoms) (Falkowski 2011; Banes 1968) (Figure 1.12). As discussed in a previous section, the type of foraminifera present in a sediment facies can provide evidence of the general geochemical regime in a locale. In addition, trace quantities of elements and isotopes are incorporated into foraminifers' shells, proving more detailed information (Hönisch 2011). Since individual foraminifera do not contain a natural growth band archive, relative abundances qualitatively indicate periods of change and stress (Bradley 1999).

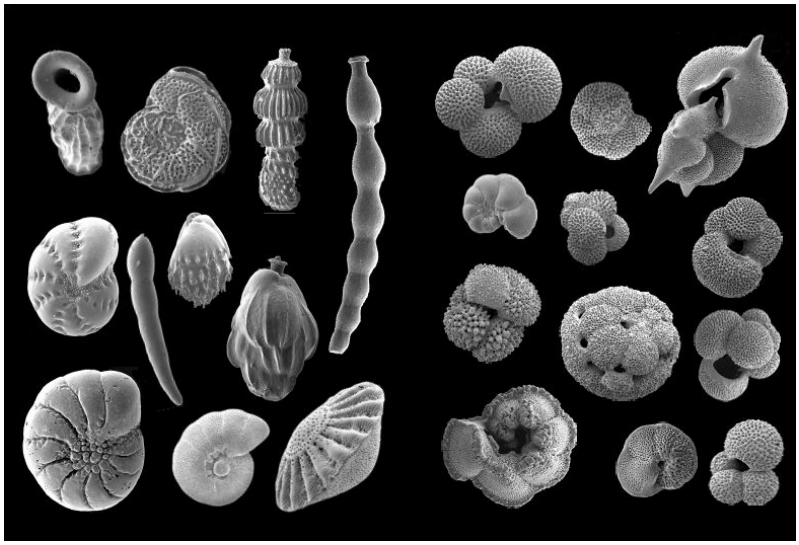


Figure 1.12: Microscope image of benthic (left) and planktonic (right) foraminifera (Paul Pearson).

Benthic and planktonic foraminifera have been shown to accurately record ambient ocean oxygen isotopes (δ18O), carbon isotopes (δ13C) and boron isotopes (δ11B), particularly as a proxy to ocean acidification, with each isotope positively correlated to pH (Katz 2010). Additionally, temperature has been connected to δ18O, Mg/Ca and Sr/Ca (Bradley 1999). However, some uncertainty in Sr/Ca relationships varies with type of foraminifera; planktonic species show a more consistent relationship (Elderfield 2002). Additionally, results have shown that the incorporation of various trace elements may be affected by relative foraminifera size, with larger individuals preferentially absorbing higher quantities (Katz 2010). Despite the inability to gain temporal resolution from individuals, foraminifera are abundant within the fossil record since the Cambrian era (Bradley 1999).

1.2.5.4. Red Coralline Algae

Red algae are marine algae distinguished by their high magnesium calcium carbonate skeleton and branching thallus appendages (Bosence 1983). These algae can exist as individuals (rhodoliths, Figure 1.13) with a mass of branching thalli and root-like holdfasts, or in crustal form with protruding branches (Adey 1970). Within the thallus appendages, coralline algae lay down growth bands, perceivable as light summer and dark winter bands (Adey 1970; Bosence 1983). Unlike coral banding which requires the use of x-ray photography, these bands can be seen under a regular light microscope (Freiwald & Heinrich 1994; Grotolli 2001).

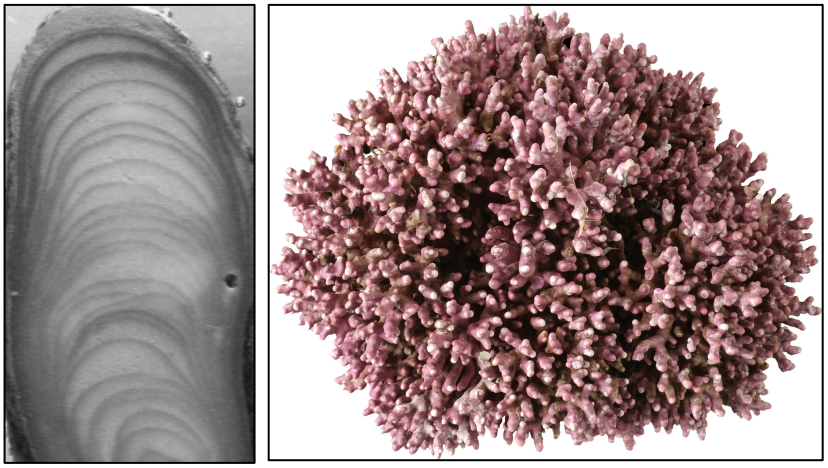


Figure 1.13: Growth bands (left) and rhodolith (right) of red coralline algae (Nick Kamenos)

Red algae beds grow at all latitudes, however they are most common at mid-to-high latitudes (Adey 1973; Nelson 2009). As with other organisms in the Plantae kingdom, red coralline algae utilize light and carbon dioxide for energy and growth (Luning 1990). In equatorial regions, the seasonal difference apparent in growth banding pairs is less pronounced than higher latitudes where temperature regimes are extreme (Kamenos 2010). Coralline algae have been shown to have a positive correlation between temperature and oxygen isotopes ($\delta^{18}O$), with some offset due to vital effects

(Halfar 2000). Strontium and magnesium to calcium ratios (Sr/Ca, Mg/Ca) have also been tested as palaeothermometers with mixed results. Studies have found Sr/Ca deposits to be positively correlated to temperature with weak significance likely due to vital effects, and therefore are not used as a proxy (Kamenos 2008). The positive correlation between Mg/Ca has been found to be significant as it is likely controlled solely by environmental temperature variations (Kamenos 2008; Halfar 2008). Carbon isotopes (δ13C) are negatively related to upwelling events and relative ambient ocean pH (Bradley 1999). The use of red coralline algae as a new biological archive is a relatively recent trend, with potential to provide data at a biweekly resolution (Halfar 2000; Kamenos 2008; Kamenos 2010).

1.2.5.5. Systematic View of Marine Proxies

The four marine proxies discussed (corals, foraminifera, bivalves and coralline algae) each differ in geographical distribution, present abundance, fossil record richness and temporal resolution. Though each proxy propagates at all latitudes, the region of optimal growth and high abundance required to produce accurate archives varies. Each proxy fills a geographical niche: corals in equatorial regions, foraminifera in benthic sediments, coralline algae at mid-to-high latitudes and bivalves at the marine-freshwater interface (Veron 1995; Hönisch 2011; Halfar 2000; Dame 2011). Temporally, the use of individual as long-term archives is preferred as it reduces uncertainty due to individual variability (Nick Kamenos, personal communication). For this reason, coralline algae and corals are favored due to the possibility of long lifespan (800 years) in contrast to the shorter bivalve lifespan (200-500 years). Apart from temporal resolution, proxy accuracy and certainty is imperative to gain an understanding of retrospective ocean system conditions. Ambient ocean temperature is utmost definite in MgCO3 concentrations in coralline algae, δ18O ratios in foraminifera and Mg/Ca concentrations in corals. Moreover, pH variability has been found qualitatively in δ18O, δ13C, Ba/Ca and δ11B in foraminifera. However, the lack of individual temporal

extent causes difficulty in producing continuous archives, rather than distinct measurements. Temporally continuous pH measurements have been estimated from $\delta^{11}B$ in corals with high uncertainty (Pagini 2005). A summarized outlook on current proxy research for corals, foraminifera, bivalves and coralline algae are summarized in Table 1.1.

The high variability in organism response to proxy measurements is a consequence of how the biological processes respond to their exterior environment, and converts trace elements and proxies into their skeletal framework (Bradley 1999). Some organisms have the ability to buffer chemical and environmental changes, potentially masking rates or amplitudes of change (Kamenos 2010). This feedback is part of a complex hierarchy of a system (single organism) within community ecosystem within the ocean. Inherent non-linearity in one part can have radiating affects throughout the system. If one ecosystem adapts to enhanced anthropogenic carbon dioxide in the equatorial surface waters by taking up more dissolved inorganic carbon through photosynthesis, the effect could be radiated by reducing the concentration of carbon dioxide transferred into the Northern Atlantic. The initial state and adaptive capacity at community level in one part of the system can alter the response elsewhere (Sastry 1999; Kauffman 1993; Kellert 1994). Such complex relationships can cause noise within the palaeoclimate proxy archive, exposing the importance of using multiple proxies (Bradley 1999).

Measurement	Corals	Foraminifera	Bivalves	Coralline Algae
$\delta^{18}O$	+ SST or SSS	+ Temperature - pH	+ Temperature*	+ Temperature*
$\delta^{13}C$	+ Light - Upwelling	- pH	Significant uncertainty from ontogenic growth	- Upwelling - pH
Sr/Ca	- Temperature	+ Temperature*		+ Temperature*
Mg/Ca	+ Temperature	+ Temperature*		$MgCO_3$ ≈ Temperature
Ba/Ca	+ Upwelling	- pH		--
U/Ca	- Temperature	--		--
Cd/Ca	+ Upwelling	--		--
$\delta^{11}B$	+ pH	+ pH		+ pH
Growth bands	Seasonality River outflow Stress	N/A		Seasonality Cloud Cover Stress
Maximum Individual Archive	800 1000 years	N/A	200 500 years	800 years
Fossil Record	Triassic	Cambrian	Cambrian	Cretaceous

Table 1.1: Various measurements and associated proxies for corals, foraminifera, bivalves and coralline algae. Stars (*) represent significant offsets from vital effects; dashes (--) represent absence of research; + signify positive correlations, and signify negative correlations. Ontogenic growth refers to the inconsistency in growth characteristics at different points in an organism's lifespan. *(Cohen 2001; McConnaughey 2008; Jones 1983; Bradley 1999; Epstein 1953; Grotolli 2001; Bradley 1999; Druffel 1997;*

Barnes 1968; Dame 2011; Lorraine 2005; Schöne 2004; Falkowski 2011; Hönisch 2011; Katz 2010; Elderfield 2002; Adey 1970; Bosence 1983; Kamenos 2010; Kamenos 2008; Halfar 2000; Adey 1973; Nelson 2009; Friewald & Heinrich 1994).

In the case of anthropogenic climate change and related ocean acidification, the geographical variability of carbon dioxide ocean uptake is important to gain an understanding of how the system is reacting as a whole. Interpreting equatorial proxies (e.g. corals) allows the interpretation of a gradual increase in the central tendency of pH (Figure 1c) and high latitudes display quasi-period variation (Figure 1b) (Caldeira 2003; Orr 2005). Furthermore, increasing variability (Figure 1d) through space is interpretable through natural CO_2 gradations at volcanic vents (Hall-Spencer 2008) and impulsive change of the central tendency at upwelling sites (Feely 2008).

1.3 Conclusions & Objectives

In summary, maerl is photosynthetic algae, composed of hard calcium carbonate lattice structures (Irvine 1977). Within their branching thalli appendages, maerl growths record high-resolution environmental data, which has recently come to light as an optimal proxy for various palaeoclimate indices (Wilson 2004; Halfar 2000). The form and structure of rhodoliths are a function of water energy and sedimentation (Foster 2000). Maerl beds serve multiple important ecosystem functions including forming the structural building blocks of coral reefs and hosting highly diverse ecosystems at all latitudes (Nelson 2009; Barbera 2003; Freiwald 1994; Bosence 1983a,b). It is predicted that maerl will record the first signs of anthropogenically-driven ocean acidification, and will be important in understanding how this process impacts the biosphere at mid-to-high latitudes (Ries 2009; Halfar 2000).

The development of palaeoclimate proxies has led to the discovery of rates and changes in climate dating before the instrumental record. Proxies vary in temporal

resolution, from the biweekly resolution in red coralline algae, to seasonal ice core resolution and 500-year marine sediment resolution (Kamenos 2008; Bradley 1999). Biological marine proxies generally have strong seasonal resolution with potential for shorter sampling intervals. Such high-resolution data sets are important in understanding the dynamics of the carbon cycle (Bradley 1999). Carbonate marine organisms are specifically suited to record the carbon cycle as they play an integral role in removing carbon from surface waters, buffering anthropogenic input, and cycling to the deep ocean, producing carbonate sediments (Ries 2011; Portner 2008; Feely 2008). Though marine carbonates have the potential to record detailed carbon processes and conditions, uncertainty arises from individual variation, local bias, ecosystem dynamics, seasonal cycles, adaptation capacities and latitudinal influences (Cohen 2001; McConnaughey 2008; Jones 1983; Bradley 1999; Epstein 1953; Grotolli 2001; Bradley 1999; Druffel 1997; Barnes 1968; Dame 2011; Lorraine 2005; Schöne 2004; Falkowski 2011; Hönisch 2011; Katz 2010; Elderfield 2002; Adey 1970; Bosence 1983; Kamenos 2010; Kamenos 2008; Halfar 2000; Adey 1973; Nelson 2009; Friewald & Heinrich 1994).

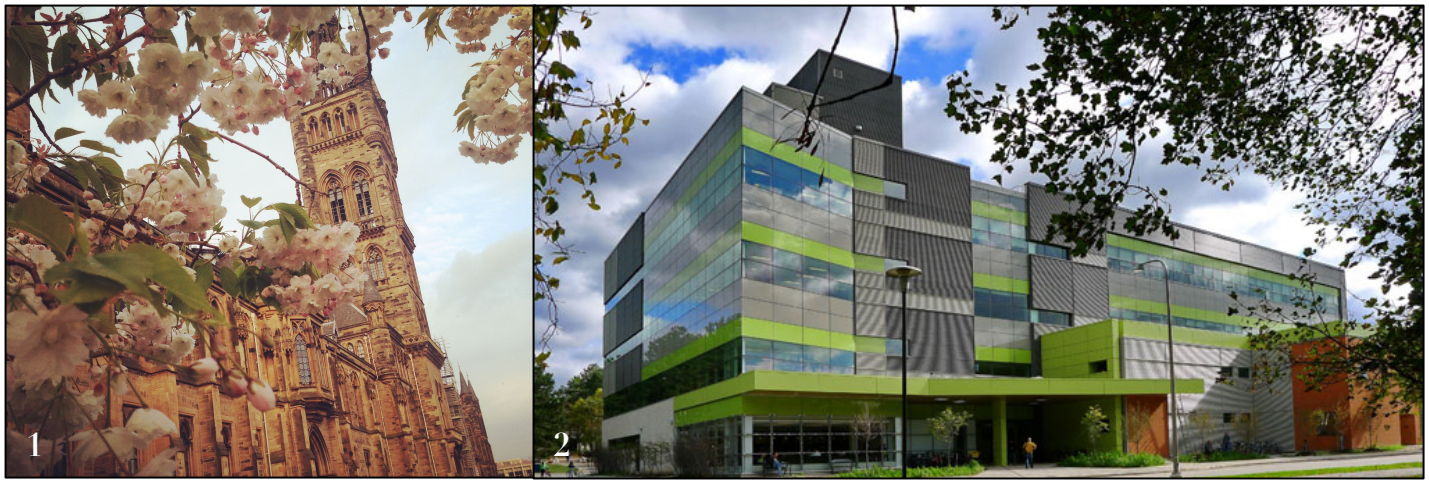
The main objective of this thesis is to determine the viability of a new marine proxy for pCO_2, utilizing high latitude red coralline algae. Rather than analyzing concentrations of chemical constituents as a direct proxy, the mechanisms of chemical bonding will be examined. In order to gain a foundational understanding of the *Lithothamnion glaciale* samples, a survey of the effects different pCO_2 concentrations and temperatures had on linear extensional growth will be completed (Chapter 3). This provided the basis for understanding photosynthesis-driven growth limitations. Subsequent analysis of relative magnesium concentration variability across seasons and treatments assist in the understanding of the process of magnesium uptake within the calcium carbonate skeletons (Chapter 4). The co-limitation of magnesium concentration and ambient temperature (Kamenos 2008) confirms the isolated

influence of seasonality and carbonate chemistry parameters. Building on the aforementioned investigations, variations of magnesium-oxygen bond strength within the calcium carbonate skeleton will be analyzed at different pCO_2 concentrations to demarcate precise impacts. The basis of the proxy development is to conclude values of Mg-O bond strengths predictable based on pCO_2 conditions.

CHAPTER 1 SUMMARY

- Red coralline algae (maerl) are a photosynthetic algae composed of a calcium carbonate
- Maerl beds found at all latitudes provide important framework for diverse ecosystems and are an imperative to the ocean carbon cycle
- Growth within a thallus (branch) shows distinct seasonal bands, suitable for use as high-resolution (biweekly) palaeoclimate proxies, since the incorporation of carbonate and other constituents are highly influenced by palaeoenvironmental parameters
- The thesis will study *Lithothamnion glaciale*, a red coralline algae species composed of high magnesium calcium carbonate; a very soluble calcite particularly sensitive to ocean acidification

CHAPTER 2
Methodology:
Sample Collection, Incubation, Processing and Analysis



1. University of Glasgow
2. University of Waterloo

CHAPTER 2 PURPOSE

The purpose of this chapter is to outline the complete research methodology used for this thesis by:

- Providing information on the study site;
- Explaining mesocosm set-up, carbonate chemistry parameters and sample selection;
- Delineating the preparation of samples;
- Outlining examination of linear extension, magnesium concentration and magnesium-oxygen bond strength, and
- Stipulating statistical analysis procedures and assumptions.

The sample collection, incubation and processing was completed at the University of Glasgow (School of Geographical and Earth Sciences) and at the Scottish Association for Marine Science.

2.1. Sample Collection

Samples of free-living *Lithothamnion glaciale* red coralline algae were collected in the summer of 2011 from Loch Sween, Scotland (56°01.99'N, 05°36.13'W) (Figure 2.1).

Figure 2.1: Location of study site (Loch Sween) on the westcoast of Scotland.

L. glaciale (Figure 2.2) are highly abundant in the western sea lochs of Scotland and surrounding the northern islands of Orkney and Shetland; sparsely located in northern Ireland, southwest coasts of Wales and England and along the northwest English coast (Jackson 2003). Loch Sween is a tidal inlet that supports dense maerl beds, whose complex calcium carbonate structures provide habitats for various marine invertebrates including echinoderms, bivalves, crustaceans and fish (SNH 2013).

2.2. Sample Staining & Incubation

Lithothamnion glaciale samples were stained with Calcein, a fluorescent dye for 24 hours at 50µm concentration, as per Brahmi 2010, to enable identification of skeletal material deposited during incubation (Figure 2.2). Following staining, thalli were grown for 24 months in the Marine Mesocosm Facility at the University of Glasgow.

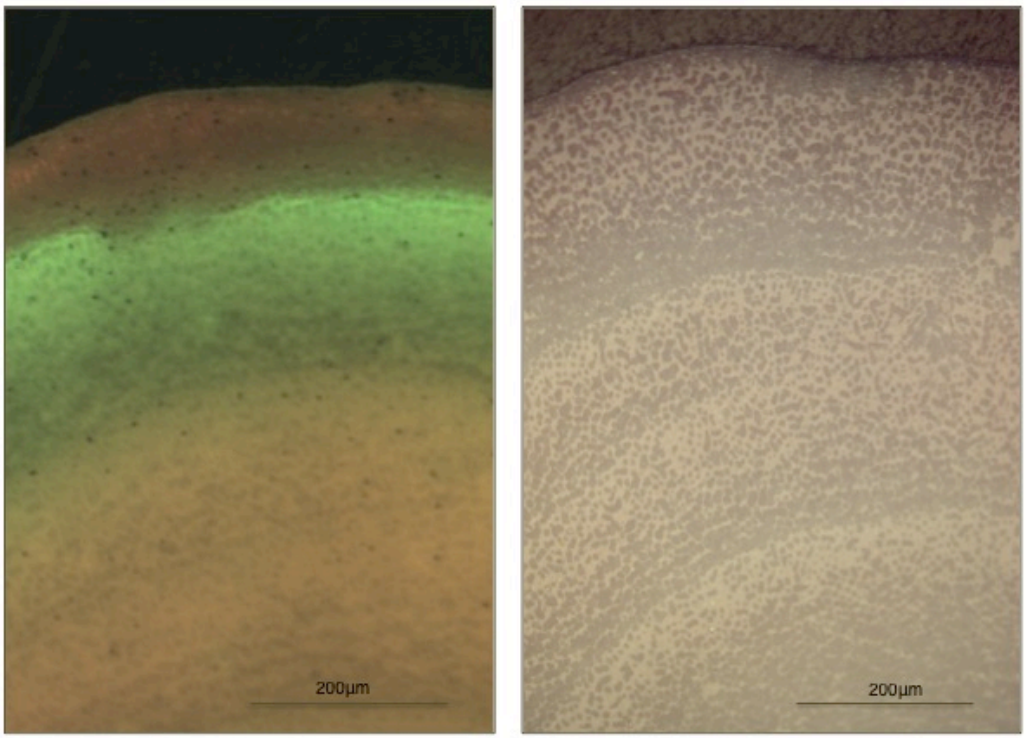


Figure 2.2: Microscope images of fluorescent stain band (left) and growth bands (right) used to identify incubation growth.

Mesocosms followed the same ambient temperatures (4.5°C during winter to 17.5°C during summer for control treatment; Chapter 4), day length and photosynthetically active radiation (90µmol photons/m²s) as that at the collection site (Rix 2012).

During incubation, thalli were exposed to one of six treatments of varying temperature and pCO_2:

1. Control pCO_2 (380µatm), ambient temperature
2. Moderate pCO_2 (750µatm), ambient temperature
3. High pCO_2 (1000µatm), ambient temperature
4. Control pCO_2 (380µatm), ambient +2°C temperature
5. Moderate pCO_2 (750µatm), ambient +2°C temperature
6. High pCO_2 (1000µatm), ambient +2°C temperature

Experimental mesocosms (Figure 2.3) were acidified by CO_2 bubbling at the desired concentrations. CO_2 concentrations were monitoring using the LI-COR 820 gas analyzers.

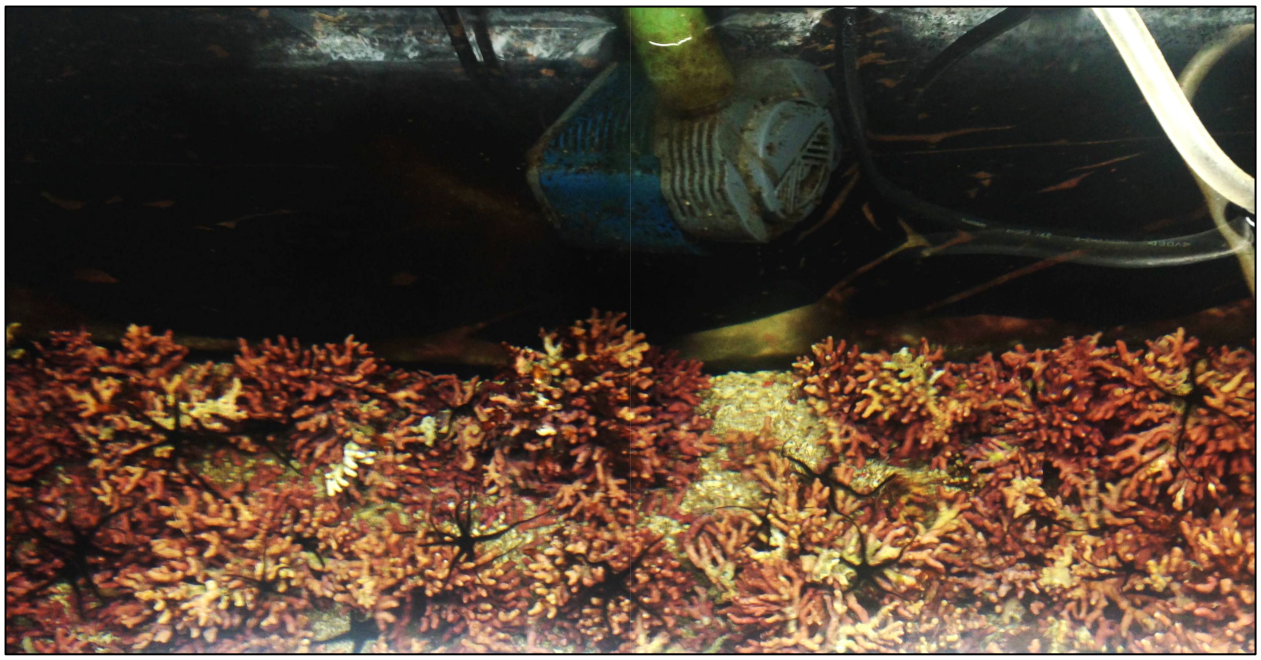


Figure 2.3: Photograph of an experimental mesocosm at the Marine Mesocosm Facility, University of Glasgow.

2.2.1. Carbonate Chemistry Parameters

During incubation, daily seawater temperature, salinity, dissolved oxygen (YSI Pro2020) and pH (Mettler-Toledo MA 235) were monitored. Every two weeks, water samples were preserved for carbonate chemistry with MgCl2 (Dickson 2007) and stored in the dark for subsequent total alkalinity (TA) and dissolved inorganic carbon (DIC) analysis. DIC was determined using a CO2 Coulometer (CM5014 v.3, UIC Ltd. At the Scottish Association for Marine Science) and methods described by Dickson 2007. TA was determined using the two-stage open-cell titration method (Dickson 2007) at the University of Glasgow. *In situ* TA, DIC, temperature and salinity measurements were used to calculate pH, pCO_2, HCO_3^-, CO_3^{2-} concentrations and calcite ($\Omega_{calcite}$) and aragonite ($\Omega_{aragonite}$) saturation states using CO2SYS (Pierrot 2006) with dissociation constants from Mehrback (1973), refit by Dickson (1987) and [KSO_4] using Dickson 1990 (Supplementary Material, Table 1).

2.2.2. Sample Preparation

Following the 24 months of growth within treatment mesocosms, branches from different *L. glaciale* individuals from each treatment were implanted in resin blocks using Buehler Epoxycure (Figure 2.4).

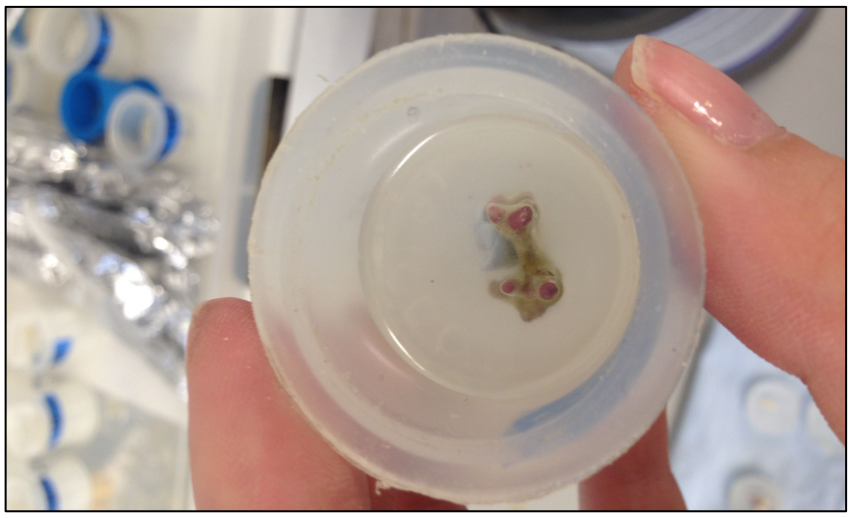


Figure 2.4: An example of a resin block with a *L. glaciale* thalli branch.

Each resin block sample was transverse sectioned using a rock saw and hand polished to expose the thalli interior along the growth axis (Figure 2.5).

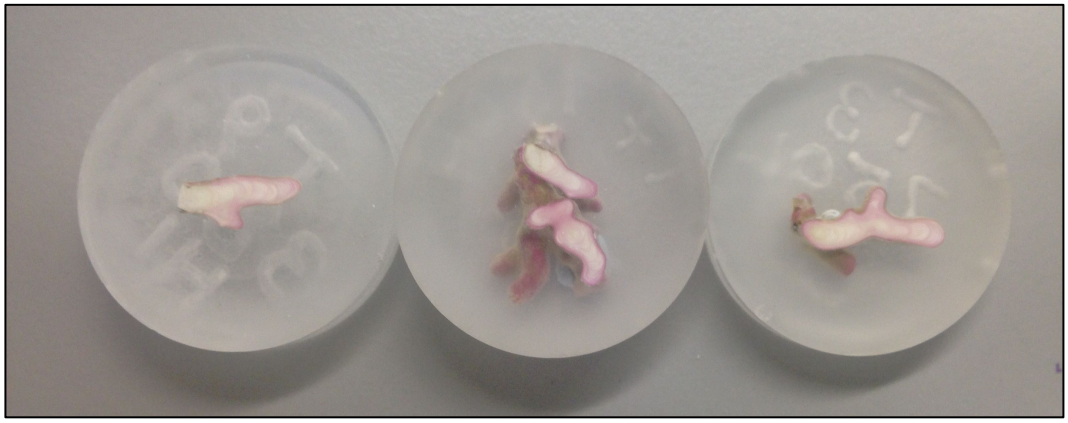


Figure 2.5: A variety of samples cross-sectioned and polished.

Growth before and after incubation treatment was identified by determining the position of the Calcein stain using fluorescence microscopy (Olympus BH-2 microscope; Figure 2.2).

2.3. Sample Analysis

2.3.1. Measured Linear Extension

Fluorescent photographs were analyzed to measure 24 months of incubation growth using GIMP v 2.8.10, GNU Image Manipulation Program. Each sample was measured in five parts of the thallus tip (parallel to the direction of growth) to reduce random errors and gain an understanding of individual growth variance (Figure 2.6). The five measurements were averaged for each individual (Supplementary Material, Tables 2 & 3).

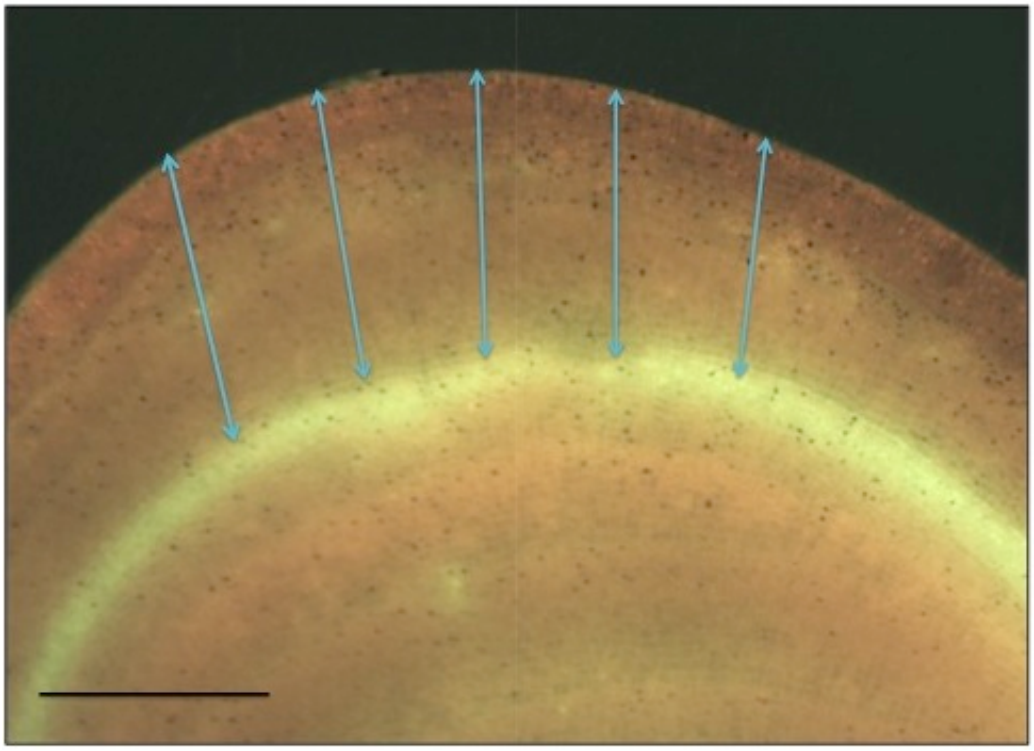


Figure 2.6: Diagram of measurement methods on a *L. glaciale* sample thallus tip. Blue arrows depict measurement pathways; length of black line represents 200µm.

2.3.2. Raman Spectroscopy

Raman spectroscopy (Renishaw inVia Raman; Figure 2.7) was conducted following the methodology of Kamenos (2013) at the University of Glasgow to determine the relative Mg concentrations (Chapter 4) and molecular bonding strength between Mg and O molecules (Chapter 5) within the high Mg calcium carbonate skeleton of *L. glaciale*. The Raman shift peak at ~1089cm^{-1} corresponds to Mg concentrations within the sample structure while the full width at half peak maximum (FWHM) is linearly correlated to positional disorder of the Mg ions within the calcite lattice and in turn bond strength between Mg and O molecules (Bischoff 1985). In *L. glaciale*, Mg is a component of the crystal lattice rather than a component of associated organics (Kamenos 2009) and thus increases in Mg-O bond strength can be attributed to

positional disorder of crystal lattice bringing Mg and O closer together via Mg ions moving out of the plane parallel to the a-axis in the direction of the c-axis (Bischoff 1985).

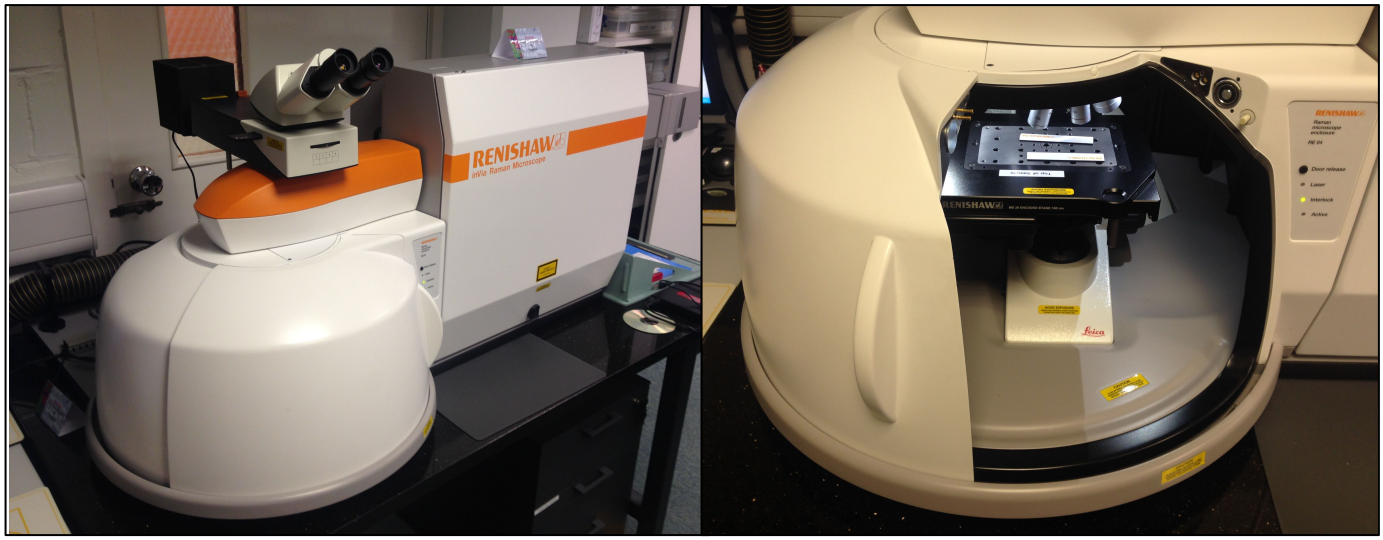


Figure 2.7: Renishaw inVia Raman exterior (left) and interior (right).

Sample Raman parameters were normalized (Normal-Inverse-Wishart distribution) to remove any differences in magnitude of starting parameters due to individual variability (some individuals naturally have stronger / weaker bond strengths than others), thus enabling direct comparison between and within treatments.

Within microscopy micrographs, each annual growth band was digitally subdivided into 6 proportional segments to give 2-month growth intervals, across summer through seasonal transition into winter (Figure 2.8). Transition from winter to spring was not considered as in *L.* glaciale this is often quite abrupt, sometimes with little skeletal calcite deposition. This procedure was conducted on micrographs of the annual growth band deposited prior to sample collection (before the Calcein stain) and the growth band subsequent to Calcein staining, which represented growth

within the mesocosm treatments. The growth prior to sample collection was also used as a control to determine any influence of sample handling on each individual.

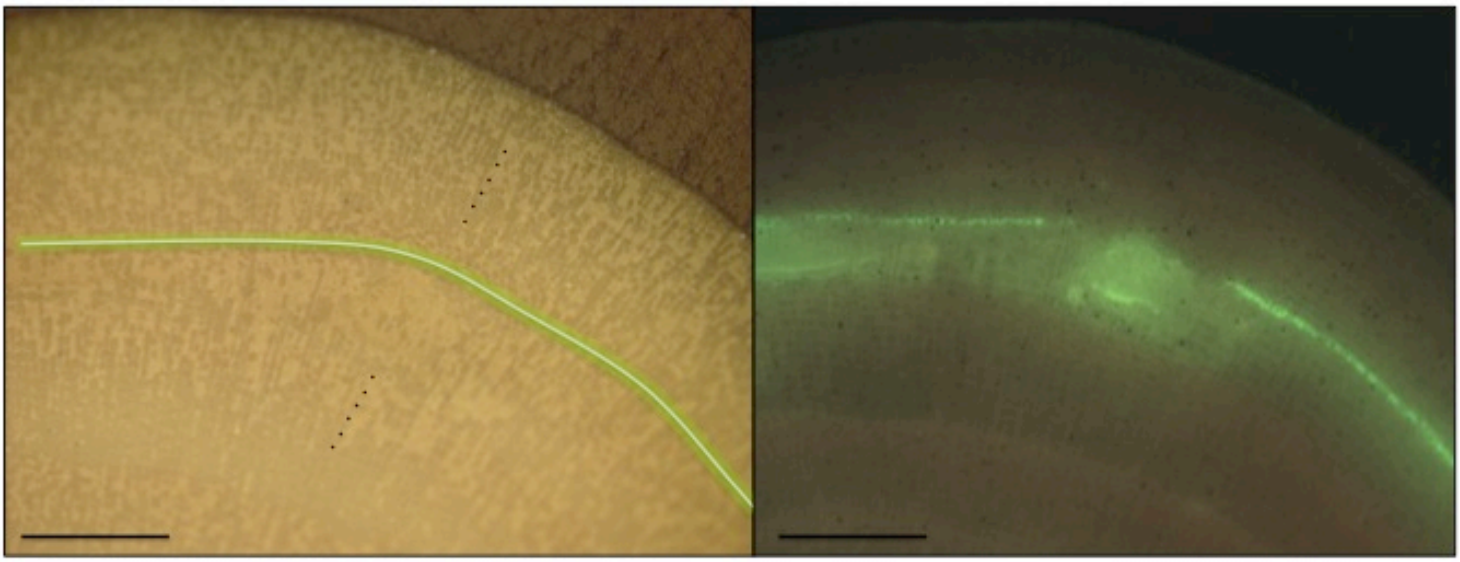


Figure 2.8: Raman spectroscopy sampling of an individual thalli (left), with fluorescent stain (right) matched on micrograph along the growth axis. Black dots represent approximate sampling sites (2 month intervals); length of black lines represents 200µm.

Mg-O bond strength and relative Mg concentrations were compared between treatments for segments of the algal growth bands that were deposited during the same season.

2.4. Statistics

2.4.1. Linear Extension

Relationships between the incubation growth and treatment group were determined using linear regression analysis (Supplementary Material, Table Series 4). Analysis of covariance (ANCOVA) was performed to determine the influence of pCO_2

(independent variable, predictor) on the outcome growth, with temperature as the covariate. Type III sum of squares was used to resolve the unequal sample sizes (n).

2.4.3 Magnesium Seasonality

Analysis of Covariance was used to determine the variability of magnesium concentration with respect to temperature and seasonal stage (Supplementary Material, Table Series 5). Once the interaction between temperature and stage was determined to be negligible, a linear model was performed between temperature, stage and magnesium concentration alone. This model was used to determine the influence of temperature (independent variable) on the magnesium concentration, with seasonal stage as the covariate. Temperature was controlled in the mesocosm treatments to parallel natural variability. The ANCOVA arrangement was performed separately for each treatment (control, 750µatm, 1000µatm) and *in-situ* measurement to determine both the influence of individual treatments and to compare experimental growth to natural growth.

2.4.3 Mg-O Bond Strength

Relationships between Mg-O bond strength and pCO_2 concentrations were determined using linear regression analysis. Analysis of covariance was performed to determine the influence of pCO_2 on Mg-O bond strength with relative Mg concentration as a covariate. PCO_2 was used as the independent variable in comparisons as treatment carbonate chemistry was controlled using CO2 bubbling. Regression analysis on normalized FWHM was used to determine the linear relationships between pCO_2 and Mg-O bond strength over the whole year of growth with relative Mg concentrations (1089 cm-1 raman peak frequency) as a covariate allowing for stage-dependent changes in lattice Mg concentrations.

2.4.4 Statistical Assumptions

All analyses were conducted in R v 3.03 and the data met all parametric test assumptions and regression analysis:

1. For each independent variable (IV), a linear relationship exists between the covariate (CV) and dependent variable (DV):
    a. Linear Extention (2.4.1): For each pCO_2 (IV), growth (DV) increases with increasing temperature (CV)
    b. Magnesium Seasonality (2.4.2): for each pCO_2 (IV), magnesium concentration (DV) decreases from summer to winter (CV)
    c. Mg-O Bond Strength (2.4.3): for each pCO_2 (IV), FWHM/bond strength (DV) decreases from summer to winter (CV)
2. Variances are homogeneous:
    a. Linear Extention (2.4.1): variability in growth is reasonably homogeneous within temperature categories (IV). Between temperature categories there is higher variability in low temperature, due to larger sample size. This was reconciled using type 3 sum of squares ANCOVA calculations.
    b. Magnesium Seasonality (2.4.2): variability in magnesium concentration is homogeneous across all samples.
    c. Mg-O Bond Strength (2.4.3): variability in FWHM is homogeneous across all samples.
3. Regression slopes (relationships discussed in (1, above) are parallel across independent variables (IV) within covariate (CV) groups.
4. Covariate is independent of dependent variable (no significant relationship):
    a. Linear Extention (2.4.1): temperature (CV) is independent of pCO_2 treatment (Supplementary Material, Table Series 4a), relationship is insignificant ($p = 0.289$)

b. Magnesium Seasonality (2.4.2): temperature (covariate) is independent of seasonal stage (Supplementary Material, Table Series 5a), relationship is insignificant ($p = 0.269$)
c. Mg-O Bond Strength (2.4.3): pre-FWHM (covariate) is independent of seasonal stage (Supplementary Material, Table Series 6a,c,e), relationship is insignificant ($p = 0.994$, $p = 0.994$, $p = 0.653$)

CHAPTER 2 SUMMARY

- Samples of *L. glaciale* red coralline algae collected in summer 2011 and stained with fluorescent dye before being incubated in marine mesocosms for 24 months:
- Growth occurred in two temperature treatments (ambient and +2°C) and three pCO_2 treatments (380µatm, 750µatm and 1000µatm), with two tanks per treatment pair
- Samples from all tanks were randomly collected for preparation (resin block cross-sectioning);
- All of these samples were examined for linear extension (Chapter 3); only those with ambient temperature were used for Raman analysis of relative magnesium concentration and magnesium-oxygen bond strength (Chapter 4 & 5).

CHAPTER 3
Linear Extension:
Impacts of environmental stressors on *L. glaciale* thallus growth

CHAPTER 3 PURPOSE

The purpose of this chapter is to assess the adaptive response of *L. glaciale* to temperature and pCO_2 changes associated with anthropogenic climate change. The relative importance of both factors in the marine biosphere will be discussed to provide a foundation for the hypothesis. The correlation between temperature, pCO2 and growth will be accomplished by analyzing the stressors singularly and collectively to delineate their proportional significance. This will be accomplished by comparing control conditions to a variety of stressor treatments to tease out thermal and hypercapnia growth thresholds over a significant acclimatization period.

3.1 Conceptual Framework

Within the marine biosphere, organisms exist at specific chemical and physical regimes, which are optimal to their survival, reproduction and growth (Schubert 2006; Eppley 1972). The precise regime is often reasonably stable within organized, well-developed marine ecosystems with some natural variability (Schubert 2006; IPCC 2007). Low trophic level, primary producers have the ability to drive ecosystem function and efficiency; influenced by a delicate balance of temperature, carbon dioxide and light utilized for photosynthesis (Schubert 2006). Of these factors, temperature is known to be a primary determinant of biological functions, organism distribution and community structure, regardless of trophic level (Eppley 1972; Brander 2005; Future Ocean; Portner 2005; Huntley 2004). For example, primary producing Lithothamnion *corallioides* red algae species are absent from the northern waters of Scotland, due to either the lack of consistently warm temperatures in summer or the crossing of a thermal tolerance threshold during winter months, reducing their ability to grow annual calcified bands (Adey 1970; Wilson 2004). Moreover, corals undergo bleaching, a bulk release of symbiotic algae and their pigments, due to thermal stress (Fitt 2001; Hoegh-Guldberg 1999). Further up the food web, bivalve species in temperate regions have much larger thermal tolerance threshold ranges than tropical species, suggesting that tropical species are less adapted to thermal variability (Compton 2007). Furthermore, oxygen transfer and

aerobic performance have been shown to be thermally dependent in a high latitude eelpout species (Portner 2007). Such examples prove that temperature variability and related geographical isotherms control biological function and population distribution differently depending on the organism (Future Ocean; Luning 1990; Portner 2005).

In addition to thermal tolerance boundaries, many marine organisms, (particularly plants and calcifying organisms) are dependent on CO_2 concentration cycles optimal to their growth and proliferation (Ries 2011; IPCC 2007). Cyclical CO_2 concentration peaks and troughs are associated with natural system processes including deep-ocean mixing, primary productivity, temperature, exterior influx and autotrophic decay (Portner 2008; Feely 2008). In the Arctic Ocean, seasonal variability in sea ice has a considerable impact on the exchange of carbon between the atmosphere and surface waters. Summer Arctic waters act as a sink when the partial pressure of CO_2 is low and as sources through polynyas and leads, when partial pressure is high in the winter (Semiletov 2004; Pipko 2002). Phytoplankton blooms have been shown to preferentially grow in areas of thin first year ice or along ice margins, following this pCO_2 pattern (Arrigo 2012). The high biodiversity, complex coral reef environments are a consequence of the relatively low pCO_2 concentrations at equatorial latitudes (Opdyke 1993;). However, in upwelling equatorial regions, pCO_2 concentration spikes reduce carbonate saturation, providing deficient chemical conditions and hindering growth of calcifying marine organisms (Feely 2008; Loucaides 2012). Additionally, natural CO_2 outlets surrounding volcanic vents demonstrate the effects of acute biological stress associated with naturally low pH conditions (Hall-Spencer 2008). Red coralline algae have been shown to distribute according to optimal pCO_2 conditions, with ecosystem abundance demonstrating a proportional relationship to distance from CO_2 outlet. In these areas, fleshy algae, which do not depend on calcification processes for structural integrity, outcompete and overtake red coralline algae (Hall-

Spencer 2008). In environments with natural pCO_2 variability, marine organisms migrate to optimal conditions, alter physiological behaviour and / or reorganize ecosystem functions (Hall-Spencer 2008; Fabry 2008; Portner 2008)

It is well documented that both atmospheric temperature and carbon dioxide concentrations are expected to concurrently increase with the influence of anthropogenic climate change (IPCC 2013). Due to the dynamic ocean-atmosphere interface, such changes will be mirrored in the ocean, highlighting the importance of understanding how organisms will react to such interdependent stressors (Sabine 2004; Martin 2009). High latitude oceans are predicted to be particularly sensitive to such thermal and chemical change as such cold waters naturally exhibit relatively low pH levels in comparison to their equatorial counterparts, due to the inherent ability for cold water to uptake higher quantities of CO_2 (Fabry 2009; Sabine 2004). In the future, this pattern will be further enhanced due to the melting of sea ice, which increases the quantity of atmosphere-ocean CO_2 exchange and reduces total alkalinity due to freshwater inputs (Steinbacher 2009; Fabry 2009).

To deal with feedbacks and regime fluctuations associated with climate change, organisms may acclimatize, alter community relationships, modify seasonal patterns, undergo physiological changes or migrate to higher latitudes and different depths to avoid reaching their thermal tolerance (Parmesan 2006). The exact combination of evolutionary/adaptive and migratory reactions may depend both on abilities of the individual organism and ecological feedbacks and cycles, each of which may modulate or complement the other (Parmesan 2006; Bradley 1999). Reaction discrepancy within communities can have radiating effects at all trophic levels. If some organisms respond to change with differing reaction magnitudes and rates, well-developed

community structures may disperse, leading to the decoupling of trophic level interactions (trophic mismatch) (Schubert 2006; Parmesean 2006).

3.2 Hypotheses

In order to assess the behavioral and adaptive response of temperature and pH changes associated with anthropogenic climate change, we will assess the singular and multiple stressors through the empirical observations of coralline algae linear extension. This will be accomplished by comparing control conditions to a variety of stressor treatments to tease out thermal and hypercapnia thresholds individually and simultaneously. It is expected that:

1. L. *glaciale* may display some acclimatization due to pCO_2 through the two year incubation period
2. The stress of increased temperatures and pCO_2 will have a greater impact on L. *glaciale* growth than pCO_2 alone
3. A relationship between pCO_2 and quantity of growth will exist

3.3 Results

3.3.1 Growth Treatment Patterns

A summary of total incubation growth for each treatment group is represented in Figure 3.1.

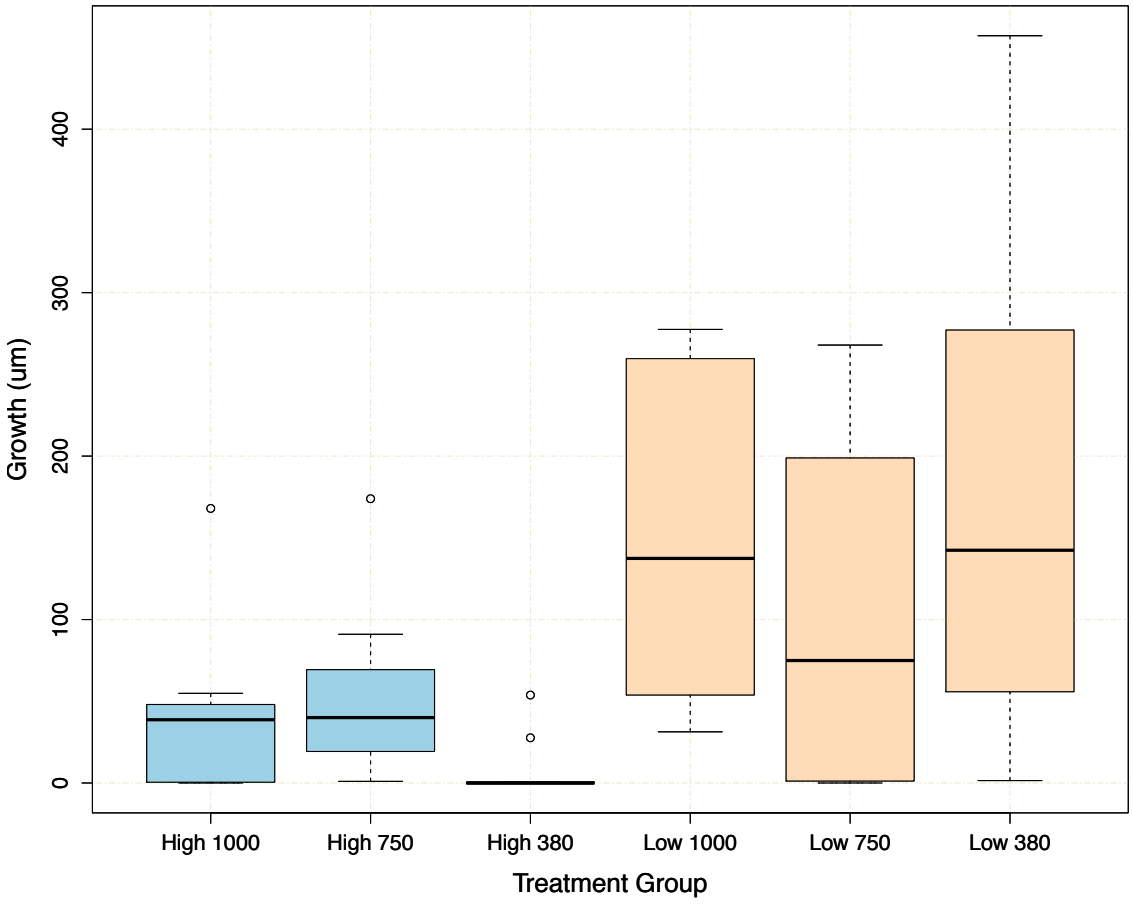


Figure 3.1: Boxplot of incubation growth results for each treatment group. Group µatm represented numerically (380, 750, 1000), temperature represented categorically (low = control (orange), high = ambient +2°C (blue)).

The results suggest that the high temperature treatments all exhibit lower median growth than the low temperature treatments (Figure 3.2).

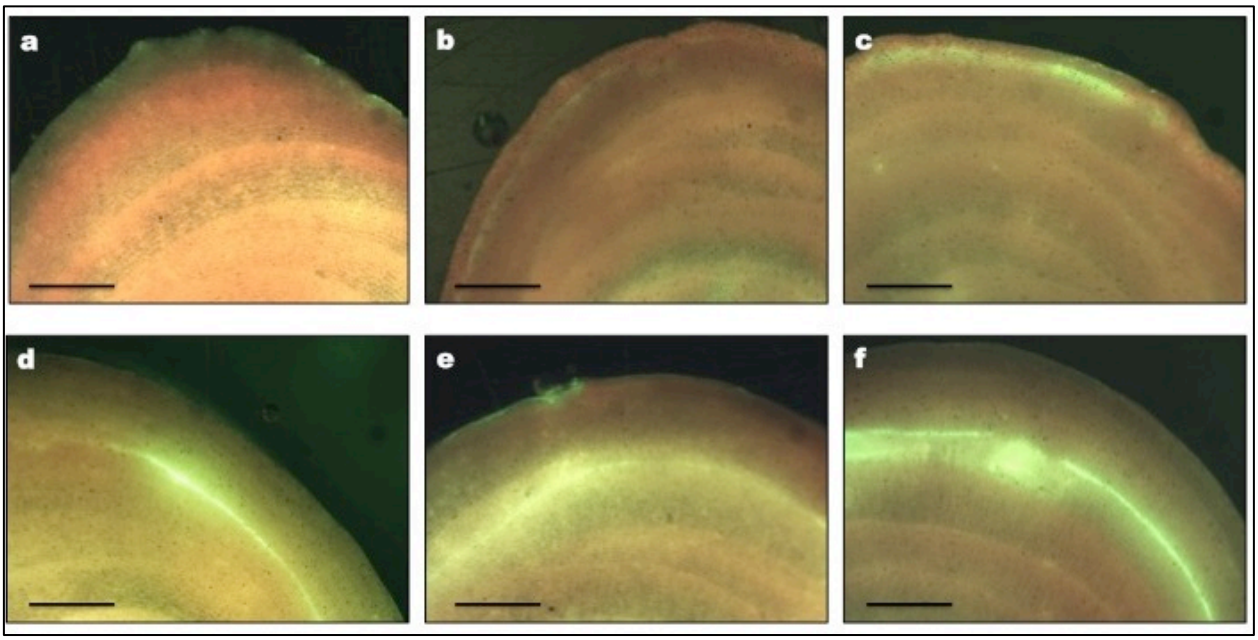


Figure 3.2: Fluorescence microscope photographs depicting an individual of each group, representing approximate median growth: (a) High temperature, 380 µatm (individual: 0 µm growth, group average: 9.09 µm ± 3.00), (b) High temperature, 750 µatm (individual: 48 µm growth, group average: 56.01 µm ± 15.53), (c) High temperature, 1000 µatm (individual: 39 µatm growth, group average: 43.38 µm ± 7.66), (d) Low temperature, 380 µatm (individual: 174 µm growth, group average: 172.33 µm ± 22.76), (e) Low temperature, 750 µatm (individual: 226 µatm, group average: 101.21 µm ± 9.84), (f) Low temperature, 1000 µatm (individual: 278 µatm growth, group average: 152.81 µm ± 15.34). Bright green line is the Calcein fluorescent dye.

While the high temperature individuals exhibit a few outliers, the total variability is considerably smaller than the low temperature treatments. The double control treatment (Low 380) had the highest total variability, ranging from zero growth to 457µm, though it demonstrates a similar interquartile range to the low 1000 group. The top ten growing individuals were all from the low temperature groups (Table 3.1), with most individuals from the 380µatm and 1000µatm treatments.

Group	Sample Number	Growth (µm)
Low 380	32	457
Low 380	38	353
Low 380	34	282
Low 1000	48	277
Low 1000	47	277
Low 380	28	272
Low 750	35	268
Low 1000	45	259
Low 750	27	226
Low 1000	52	202

Table 3.1: Top ten incubation growth individuals from all treatments.

3.3.2 Individuals with No Growth

With exception to the Low 1000 (low temperature, 1000 µatm CO_2) group, all other treatments exhibited individuals with virtually zero growth (Figure 3.3). The group with the highest proportion of no growth individuals was the high temperature, control pCO_2 group, with a value of 78%.

3.3.3 Analysis of Covariance / Multiple Stressors

The analysis of covariance test indicated a significant negative relationship ($p = 0.001$) between treatment temperature and incubation growth (Supplementary Material, Table Series 4). No significant relationship was found between growth and carbon dioxide concentration, and no interaction between temperature and pCO_2.

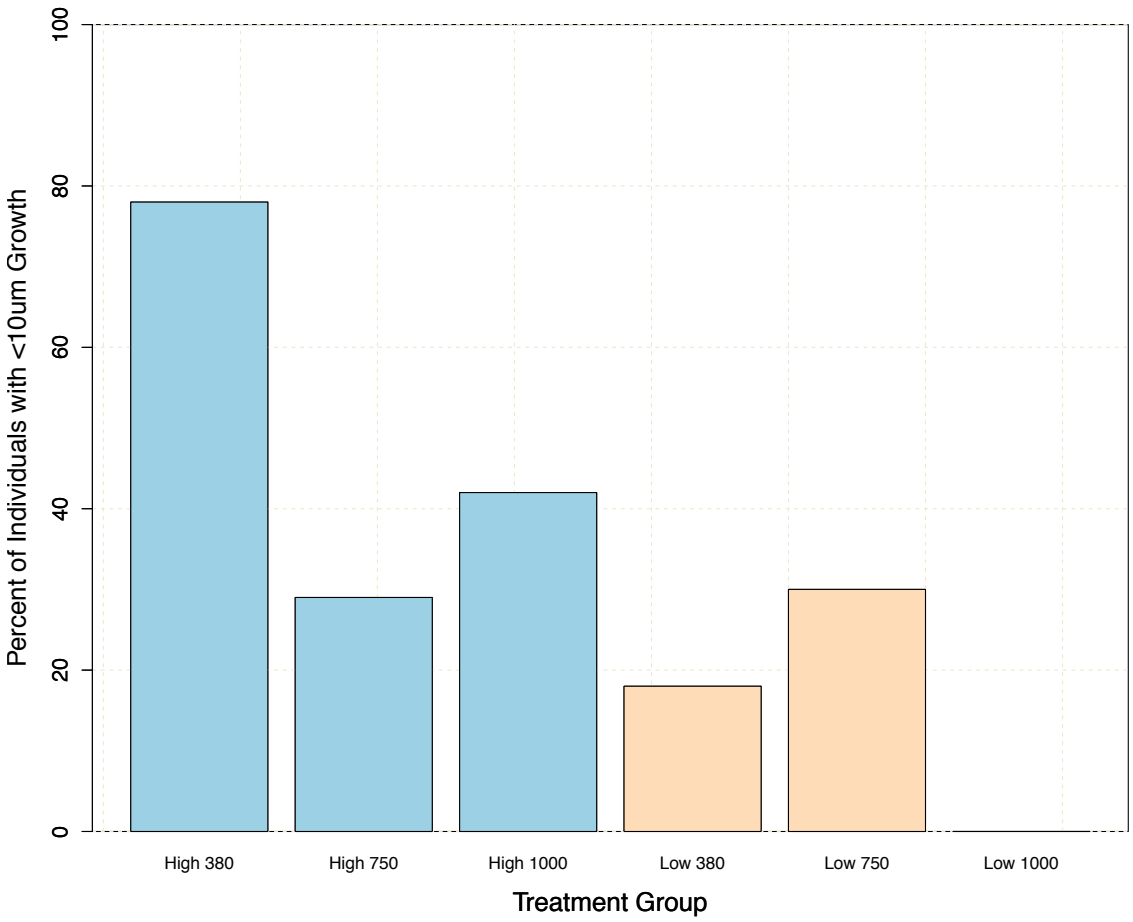


Figure 3.3: Histogram of the percentage of individuals with <10µm of growth post-treatment. Group µatm represented numerically (380, 750, 1000), temperature represented categorically (low = control (orange), high = ambient +2°C (blue)).

3.4 Discussion

3.4.1 Temperature and Growth

The growth results of the *L. glaciale* samples showed a strong relationship with temperature of incubation. The temperature controlled the consistency of growth within the samples, with the samples grown in higher temperature exhibiting a

reduction in total linear extension variability. Though the higher temperature samples grew more consistently across samples, the growth was significantly and distinctly lower. These results suggest that temperature strongly drives the ability for *L. glaciale* to grow. Since *L. glaciale* is a high latitude species, growing closer to the lower bounds of its thermal threshold, theory suggests that higher temperatures would likely enhance photosynthesis and growth (Martin 2009). However, the increase in temperature may have stimulated winter growth, when *L. glaciale* lay down few, heavily calcified cells and stressed summer growth, decreasing photosynthesis efficiency, rather than stimulating it. The high temperature may have limited the process of carbon dioxide assimilation, creating an imbalance in process of carbon metabolism (Weis 1988).

3.4.2 Carbon Dioxide Concentration and Growth

There was no significant relationship found between growth and ambient pCO_2 conditions. However, total variability in growth decreased in proportion to ocean acidification severity, with the control treatment demonstrating the highest variability. The median growth was approximately equivalent between the control and high ocean acidification treatments, with a slightly smaller median in the moderate treatment. These result deviate from Ragazzola (2012), who found a significant reduction in total growth between the control and high (1018µatm) pCO_2 treatments of *L. glaciale*. However, Ragazzola maintained a constant temperature (eliminating the complex influence of seasonality on *L. glaciale* growth) and maintained incubation for only 3 months (reducing the ability of the algae to acclimatize).

Our findings suggest that pCO_2 enrichment did not stimulate photosynthesis over the control treatment, but may have regulated carbon metabolism. Though the quantity of

growth was not significantly different between the control and ocean acidification treatments, the quality of growth (calcification stability) may have been affected.

3.4.3 Covariance of Temperature and pCO_2

The combined stress of enhanced temperature and pCO_2 encouraged linear extension growth of the *L. glaciale* samples. The median growth of high temperature samples was approximately equivalent for the moderate and high ocean acidification treatment, and was higher than the control, which had little to no growth. The additional stressor of temperature altered the proportional influence at the threshold of moderate ocean acidification; growth being lower without enhanced temperature. However, the clarity of the proportional pCO_2 control is clouded by the strong temperature influence on growth.

3.5 Conclusions

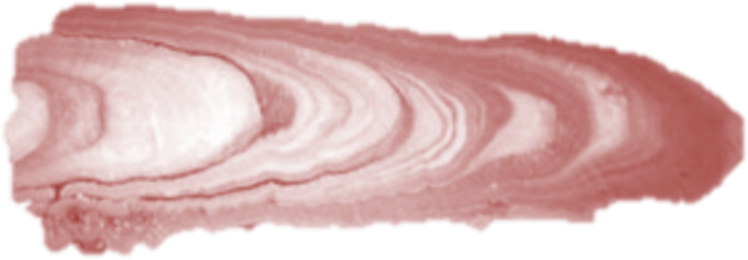
The growth of high latitude, *L. glaciale* red coralline algae have been shown to be highly dependent on ambient temperature, with a significant growth reduction experienced by the +2°C samples. As *L. glaciale* living along the west coast of Scotland are living on the low end of their thermal thresholds, the negative influence of temperature alone deviates from photosynthesis principles. A similar experience conducted on tropical species, would yield stronger results (Ries 2011). Since *L. glaciale* are common at high latitudes, they will experience the first signs of a warming ocean in addition to diminishing pH levels. Our results suggest that *L. glaciale* may have the ability to acclimatize to ocean acidification conditions in terms of growth, but the affect on calcification and structural integrity is beyond the scope of this study. While CO_2 enrichment may not significantly affect *L. glaciale* as a high latitude species, temperature enhancement, associated with anthropogenic climate change may have a strong impact. Though *L. glaciale* may have the ability to grow through

photosynthesis fertilization and utilization of HCO_3 (via CO_2 conversion) at high pCO_2, the efficiency of calcification processes that bind the high Mg calcite structure are imperative. A reduction in structural integrity via reduced calcification of *L. glaciale* may have impacts on the role of maerl communities at high latitudes, which provide a complex framework for biodiverse ecosystems.

CHAPTER 3 SUMMARY

- Temperature, light and pCO_2 are important factors influencing the growth of photosynthetic marine organisms, including red coralline algae; effecting marine organism distribution and ecological function
- *L. glaciale* grown at elevated temperatures (+2°C) exhibited significantly lower growth than those growth at ambient temperatures, regardless of the pCO_2 concentration
- Results suggest that temperature is a more important driver of growth than pCO_2; the increase in temperature may have stimulated winter growth and stressed summer growth
- The reduction in available carbonate ions as a result enhanced pCO_2 may have been overcome by *L. glaciale* through the utilization of bicarbonate for calcification
- The ambient extremes in temperature and pCO_2 controlled in this study deviates from other studies (eg. Ragazzola 2012) which maintain constant conditions, causing deviations in results; this proves the importance of considering the complexities of seasonality for high latitude species

CHAPTER 4
Magnesium Concentration:
Structural variability of the high Mg calcium carbonate structure of *L. glaciale*



CHAPTER 4 PURPOSE

The purpose of this chapter is to delineate the seasonality of magnesium incorporation into the calcium carbonate structure of *L. glaciale* to provide a foundation for subsequent palaeoclimate proxy testing using magnesium-oxygen bond strength. Magnesium incorporation will be discussed in terms of:

1. Oceanic Mg:Ca dynamics and palaeoenvironmental consequences
2. Structural complexities of incorporation into a calcite framework
3. Influence of temperature and pCO_2 on relative magnesium concentration in *L. glaciale* samples

4.1 Conceptual Framework

A wide range of marine organisms depends on the balance of carbonate chemistry parameters for calcification of their shells and hard structures. Calcified parts make up as much as 80-90% of a carbonate organisms' biomass (Bilan 2001). Marine carbonate organisms are composed of variable forms of calcium carbonate: calcite, aragonite and magnesium calcite. These differ in terms of crystal structure and chemical compounds, which impact their relative stability in seawater (Berner 1975). The most stable form is calcite, has a trigonal-hexagonal crystal structure composted of horizontal planes of equilateral CO_3 bonds between planes of calcium. The less stable, aragonite is composed of the same chemical formula as calcite, but differs in its orthorhombic crystal structure (Morse 2007). The least stable, magnesium calcite possesses the same general crystal arrangement as calcite, with substitutions of magnesium for calcium within the structure (Morse 2007; Berner 1975) and is the focus of this thesis.

High magnesium calcite red coralline algae are composed of approximately 8 to 29 mol % of $MgCO_3$ within their $CaCO_3$ crystal structure (Chave 1954), with L.

glaciale species composed of 13 to 25 mol % (Kamenos 2008). The incorporation of Mg into the calcite lattice occurs as a result of the ambient ocean Mg:Ca concentration ratio, rather than the absolute magnesium concentration (Ries 2006; Berner 1975). The reaction, in which magnesium ions are substituted for calcium ions within calcite, is endothermic, and therefore the magnesium concentration parallels ambient temperature variability (Katz 1973). The thermodynamic control on magnesium uptake in a pure crystal has been found to be exponential within a given temperature range (0 to 30°C)(Chave 1954), however this driving factor is highly variable in biogenic samples, and has been found to be species dependent (Lea 1999). In red coralline algae, the process is principally driven by abiotic processes (Berner 1975; Ries 2006), without strong biological control on incorporation (Kamenos, 2009). For this reason, measurements of magnesium concentration and related structural characteristics can be used as a proxy for environmental parameters at the time of deposition (Kamenos 2008). This relationship is valid for the Quaternary period, when Mg:Ca ratios have been relatively constant due to the long-term residence time of these cations within the ocean (Lear 2002). In deep time, past the Quaternary, Mg:Ca ratios were more variable, dividing the ocean into calcite and aragonite ocean modes. During calcite ocean modes, where Mg:Ca ratios are high, the globe is in a interglacial state; glacial conditions are reflective of low Mg:Ca ratios during aragonite ocean modes (Sandberg 1983; de Villiers 1999)(Figure 4.1). Such divergences in ocean conditions and ratio concentrations are a function of freshwater input, mineral emission from ocean trenches and carbonate precipitation removal (Dickson 2002). Additionally, high Mg:Ca ratios have been correlated with increased sea level and volcanism and CO_2 concentrations (Sandberg 1983; Wilkinson 1985). Presently, the Mg:Ca concentration is estimated to be approximately 5.2 mol/mol Mg:Ca,

placing the contemporaneous climate in a *greenhouse* state (de Villiers 1999; Sandberg 1983). Taking into consideration the current, stable Mg:Ca oceanic ratio, it is assumed that the calcified concentration within marine carbonates is mainly consequence of ambient temperature (Lear 2002).

Some marine carbonate organisms incorporate magnesium heterogeneously into their structure, with some areas preferring magnesium uptake, known as domains (Moberly 1968). For example, echinoderms exhibit different concentrations of magnesium within their plates, teeth and spines (Chave 1954). Red coralline algae however, exhibit more homogeneity, though random, uptake of magnesium (Ries 2006; Bischoff 1985).

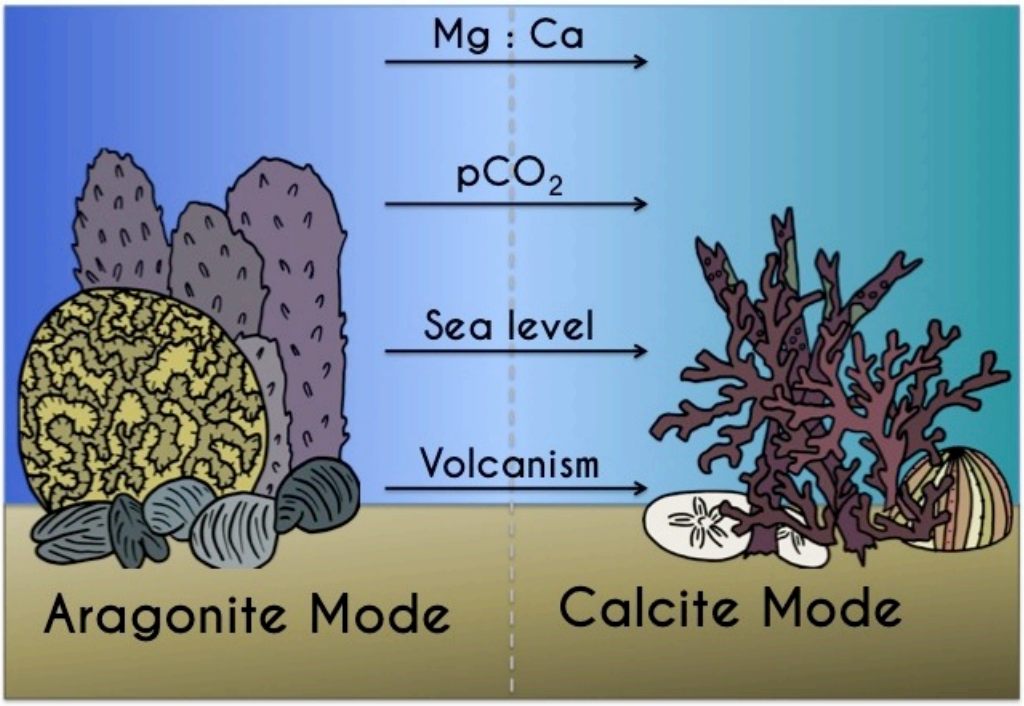


Figure 4.1: Characteristics of Aragonite and Calcite ocean modes. Arrows represent increasing quantities of given attribute. (Created by Pauly using information from Berner 1975, Lear 2002, Sandberg 1983, Ries 2005)

Within the high magnesium calcite arrangement, magnesium is substituted randomly in biogenic structures (Bischoff 1983) (Figure 4.2).

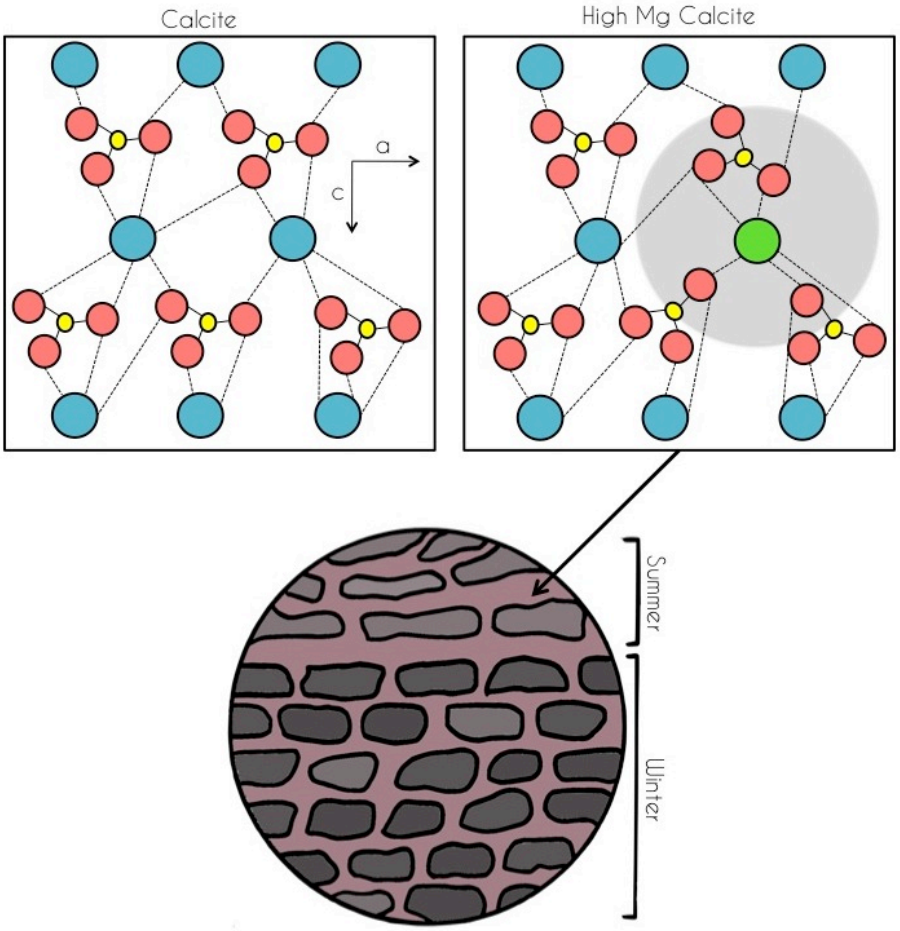


Figure 4.2: An example of calcite and high magnesium calcite (HMC) chemical structure (top). Elements represented as colours: blue = calcium, red = oxygen, yellow = carbon and green = magnesium. Lines represent bonds, and grey shadow delineates carbonate ion tilting out of a-axis towards c-axis. Bottom: location of HMC calcification within the cell walls of a red coralline algae. (Created by Pauly using information from Ragazzola 2012, Kamenos 2010, Bischoff 1985, Morse 2007)

In this substitution, the magnesium ions have a smaller cation size than calcium ions. For this reason, the crystal structure is slightly altered to account for the decreased cation size, which results in a smaller but stronger bond with oxygen (Mg-O) than the larger, weaker Ca-O bond (Bischoff 1985; White 1947). In areas of magnesium cation uptake, where cell volumes decrease, carbonate ions are forced to tilt out of their planes to compensate for the impurity (Bischoff 1985; Dauphin 1999), as discussed in the subsequent chapter.

Based on the assumption that red coralline algae incorporate magnesium into their (high magnesium) calcite structure randomly and homogeneously, without vital effects, within a time of constant ocean mode, we will measure this quantity as a proxy for temperature patterns. This outcome will provide a foundation for understanding the influence of seasonality and seasonal stages on the L. *glaciale* samples at different pCO_2 environments.

4.2 Hypotheses

The Raman Spectrometry measurements do not provide a quantitative magnesium concentration value, but rather a relative concentration and insight into structural behaviour (Bischoff 1985).

It is expected that:

1. Relative magnesium concentrations will parallel temperature patterns across seasons and relate to defined seasonal stages
2. Magnesium concentrations and seasonality patterns will be marginally affected by ocean acidification treatments

4.3 Results

4.3.1 Mg Concentration Seasonality

All samples, both *in-situ* (growth prior to incubation) and in treatment (growth during incubation), exhibited a seasonal relationship in relative magnesium concentration from summer, through transition into winter (Figure 4.3).

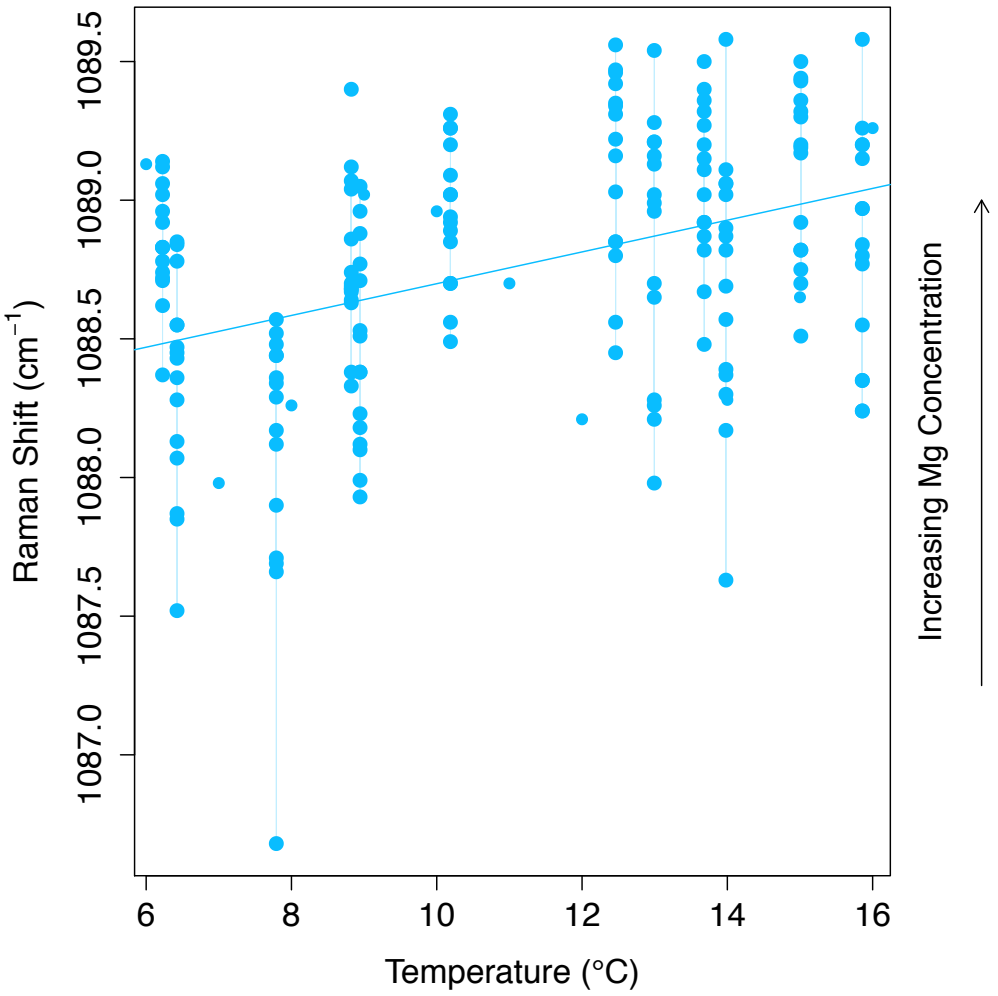


Figure 4.3: Linear relationship between relative magnesium concentration (Raman shift) over temperature.

Average summer concentrations were always found to be higher than average winter concentrations, which follow ambient temperature patterns (Figure 4.4). While Mg concentrations were lower in all treatments after laboratory incubation (Figure 4.4) a significant difference only occurred in the 1000µatm pCO_2 treatment ($F = 21.96$, $df = 1$, $p < 0.001$). This difference generally occurred during the less optimal transition and winter growing conditions.

According to the analysis of covariance (Supplementary Material, Table Series 5) results, no significant interaction occurred between temperature and stage to influence the magnesium concentration. A significant relationship between temperature and magnesium concentration was found for all treatment measurements (380: $F = 34.39$, $df = 1$, $p = 0.01$, 750: $F = 38.52$, $df = 1$, $p = 0.01$, 1000: $F = 62.55$, $df = 1$, $p = 0.01$) and *in-situ* measurements ($F = 17.60$, $df = 1$, $p = 0.001$). A relationship between seasonal stage and magnesium concentration was found in all treatments, but only significant in the control ($F = 10.77$, $df = 1$, $p = 0.05$) and *in-situ* ($F = 5.28$, $df = 1$, $p = 0.01$).

The co-variation of temperature and seasonal stage differed between *in-situ* and incubation measurements, with a stronger correlation from the natural *in-situ* environment ($F = 25.59$, $df = 1$, $p = <0.001$) than the mesocosm environment ($F = 9.32$, $df = 1$, $p = 0.05$). The temperature data parallels these results, as the temperature change occurs marginally faster *in-situ* (Figure 4.4).

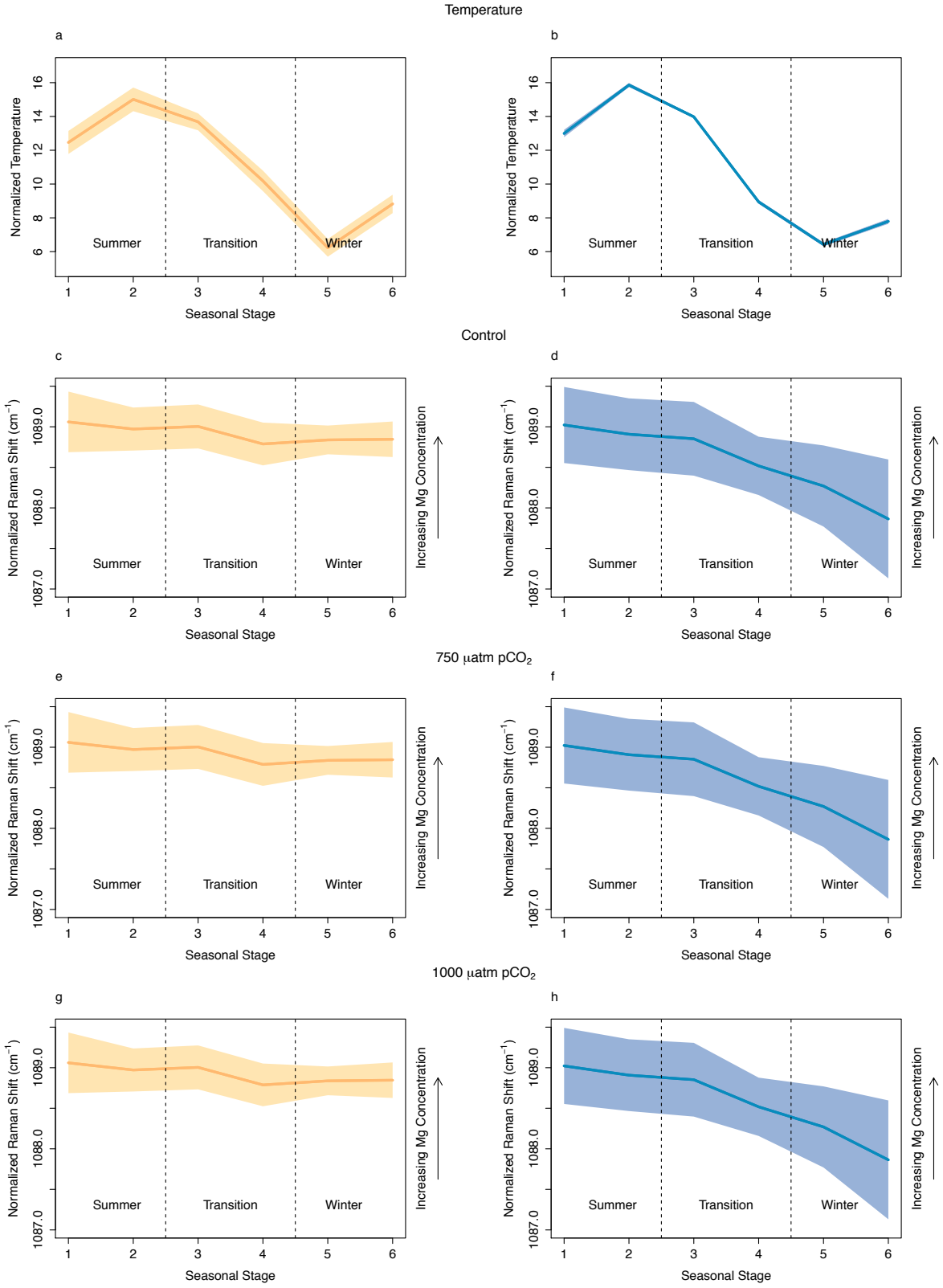


Figure 4.3: A record of ambient temperature for one full year for (a) *in-situ* and (b) incubation environments. Average pre-treatment seasonality in raman shift (cm^{-1}), indicative of relative magnesium concentration, for (c,d) 380pCO_2, (e,f) 750 pCO_2 and (g,h) 1000 pCO_2 treatments. The orange graphs (c,e,g) indicate measurements obtained from growth layers developed prior to incubation, and the blue graphs (d,f,h) indicate those developed during treatment. Shaded areas denote standard error.

Incubated samples demonstrated a stronger seasonal signal, with a greater difference between the seasons. The average summer-to-winter relative magnesium concentration difference was 0.75cm^{-1} raman shift for incubated samples, and 0.35cm^{-1} raman shift for *in-situ* sample measurements (Figure 4.4). Additionally, the incubated sample measurements had a larger standard error in seasonal variability than the *in-situ* measurements.

4.3.1.1 Treatment Influence

The significance of the relationship between temperature and magnesium concentration increased with increasing pCO_2 (380: p = 0.01, 750: p = 0.008, 1000: p = 0.004) for incubated growth. The relationship significance was the highest for *in-situ* growth of all samples (p = 0.001).

4.4 Discussion

The seasonal change in Mg concentrations (Figure 4.4) is due to a positive correlation between Mg concentration and in-situ temperatures within coralline algae driven by the abiotic replacement of Ca by Mg ions within the calcite lattice at higher temperatures (Kamenos 2008; Kamenos 2009). These predictable seasonal patterns underline the importance of either comparing high Mg calcite that was deposited at the same time of year for different pCO_2 treatments or allowing for the concentration of Mg present. In addition to the correlation between temperature and Mg concentration, the correlation between seasonal stage and magnesium concentration proves the viability of the summer, transition and winter seasonal stage delineation.

The average reduction in Mg concentrations in all treatment samples is likely due to acclimatization to new conditions. However, as a significant reduction only occurred in the high ocean acidification treatment, particularly during the winter, this may be due to the combined effects of the sub-optimal seasonal growing conditions stress due to high ocean acidification. These conditions have likely affected the ability for the *L. glaciale* to calcify, and thereby uptake Mg into their calcite skeleton. Since the significant differences only occurred in the high ocean acidification treatment, the handling impacts as a driving variable for observed Mg concentration changes can be excluded. However, these results may be influenced by the slight difference in seasonal stage to temperature relationship. The seasonal transition phase occurs marginally faster and winter temperatures average colder *in-situ*. The treatment samples warmer winter growing conditions, may have slightly counteracted the Mg concentration reduction. The influence of moderate and high ocean acidification treatments on magnesium uptake and seasonality was evident when comparing these measurements to the control. Though the ocean acidification treatments exhibited an equivalent significance between magnesium concentration and temperature, a significant relationship between seasonal stage and magnesium was lacking. As discussed, this may be due to the suboptimal pH conditions, reducing calcification ability. Based on results in Chapter 3, the *L. glaciale* samples continued to grow at moderate and high carbon dioxide concentrations; this growth was likely less heavily calcified and more variable than the control treatment.

4.5 Conclusions

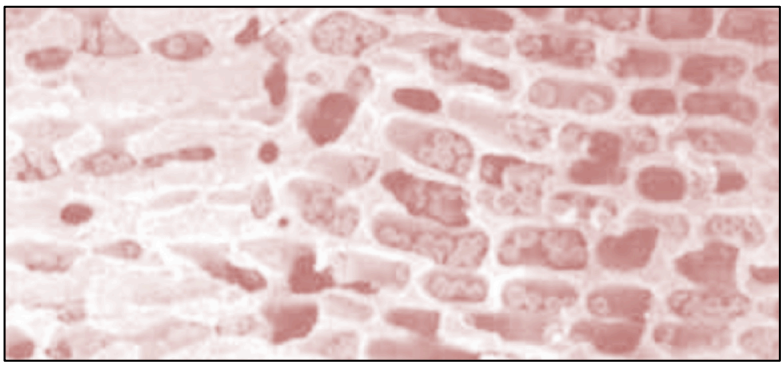
Our results indicate that uptake of magnesium cations into the high magnesium calcite structure of *L. glaciale* is significantly driven by ambient temperature

conditions both *in-situ* and in incubation. This relationship was maintained through the moderate and high ocean acidification treatments, with a diminutive reduction in significance. However, the correlation between seasonal stage and magnesium concentration is weak in the high ocean acidification treatment during the winter, suggesting that though the temperature signal remains, high pCO_2 influences the calcification and possibly the efficiency of magnesium uptake. Nonetheless, this influence is inconsequential in utilizing magnesium concentration as a proxy for ambient ocean temperature as demonstrated by Kamenos (2008).

CHAPTER 4 SUMMARY

- *L. glaciale* red coralline algae are composed of the least stable form of calcite: high magnesium calcium carbonate
- The incorporation of magnesium (over calcium) into the calcium carbonate framework of *L. glaciale* alters the crystal structure based on the smaller molecule size of magnesium relative to calcium
- The incorporation of magnesium into marine calcifying organisms is a function of ambient oceanic Mg:Ca, is thermodynamically driven and organism dependent
- In *L. glaciale* this process is abiotically-driven and therefore accurately reflects ambient conditions; results prove a significant relationship between magnesium incorporation and temperature in the incubated samples, regardless of pCO_2 concentration
- These results are required when examining magnesium-related bonding within *L. glaciale* as they prove the importance of considering temperature (or season) as it has an overriding influence on structural dynamics via magnesium incorporation

CHAPTER 5
Mg-O Bond Strength:
Testing the utilization of a new marine pCO_2 proxy



CHAPTER 5 PURPOSE

The purpose of this chapter is to test the viability of a new ocean pCO_2 proxy based on structural factors rather than chemical concentrations. The influence of carbonate chemistry on the structural integrity of red coralline algae and the current state of pCO_2 proxy development will be discussed.

The correlation between ambient pCO_2 and *L. glaciale* magnesium-oxygen bond strength will be examined by analyzing divergences across three ocean acidification treatments (control, moderate and high) over two years. From the results, an approximate linear model will be developed and discussed in terms of seasonal variability, temporal resolution and proxy stability.

5.1 Conceptual Framework

The ocean carbon cycle is driven by atmospheric exchange of dissolved inorganic carbon, which controls pH, local equilibrium and carbon buffering capacity (Caldeira 2003). Recent climate change generated by anthropogenic drivers on the Earth system has amplified this exchange (IPCC 2013; Caldeira 2003). In particular, CO_2 emissions associated with land use changes and combustion of fossil fuels have increased atmospheric CO_2 concentrations from 275 µatm in 1700 (Neftel 1994; Keeling 1993) to 400 µatm today (Tans 2014) with the oceans absorbing 25-30% of anthropogenic CO_2 released into the atmosphere (Bates 2012). The dissolved carbon dioxide reacts with water to form carbonic acid and as CO_2 levels increase at the atmosphere-ocean interface, the carbonic acid is reduced to hydrogen ions and bicarbonate. This secondary process reduces carbonate saturation state and pH levels, effectively acidifying the ocean, a process called ocean acidification (OA)(Doney 2009).

Such changes in carbonate saturation states can have impact on the marine biosphere. As carbonate ions become limiting, fewer are available for the growth and propagation of calcifying marine organisms (Feely 2009), including coralline algae (Kamenos 2013). Although many marine organisms are expected to be

adversely affected by ocean acidification (Hall-Spencer 2008; Beaufort 2011; Rodolfo-Metalpa 2011), inter-species variability in susceptibility exist (Fabricius 2011; Inoue 2013; Ries 2009). While OA experiments have been undertaken both in situ (volcanic vents, pelagic mesocosms) and in the laboratory to access marine organismal vulnerabilities (Dupont 2013), the rates of change in carbonate chemistry are often faster than those likely to be experienced during OA providing unrealistic scenarios for understanding organismal change (Kamenos 2013).

The Paleocene-Eocene Thermal Maximum (PETM) is an event often utilized as an analogue for current anthropogenic climate change (Zachos 2005). During the PETM, large quantities of carbon (as methane) were released and dissolved into the oceans, causing mass OA (Penman 2014). The PETM was marked by a significant negative δ13C excursion (Zachos 2005). Current anthropogenically-driven CO_2 release is projected to be of similar magnitude, but higher rate, than the PETM (Zachos 2005). More rapid OA precludes ocean mixing and carbon dissipation (Zachos 2005) enhancing organism stress (Kamenos 2013). Thus such palaeoclimate archives can also be used to assess rates and influences of acidification change in recent centuries.

High-resolution reconstructions of oceanic pH or pCO_2 are used to understand changes in the carbon cycle, including OA (Hönisch 2012). Recent reconstructions have focused on boron concentrations, dependent on the proportional concentrations of boric acid to borate ions, to reconstruct seawater pH patterns. For example, boron isotopes have been used to reconstruct PETM process (Penman 2014; Babila 2014), the Miocene Climatic Optimum (Foster 2012) and the last deglaciation (Henehan 2013). In addition to boron isotopes, $\delta^{13}C$ isotopes

have been negatively correlated to pH in foraminifera and coralline algae in addition to $\delta^{18}O$ and Ba/Ca isotopes in foraminifera alone (Katz 2010; Bradley 1999; Elderfield 2002). Proxy development utilizing foraminifera is viable to produce records for deep ocean sediments, which undergo an exceptionally divergent carbonate chemical regime than shallow water environments (Bradley 1999; Prentice 2001). For this reason, proxy development employing corals and coralline algae fill the niche of paleoclimate reconstruction on continental shelves; with coral research commonly constrained to equatorial regions and coralline algae are frequent in temperate and Arctic regions (Vernon 1995; Adey 1973; Nelson 2009.

Red coralline algae are high-resolution palaeoenvironmental proxies with wide geographical distribution (Heinrich 1995), long-lifespan (Foster 2001), seasonal growth banding (Kamenos 2008), lack of ontogenic growth (Halfar 2011) and, in some species, reduced structural and physiological susceptibility to changes in carbonate chemistry (Kamenos 2013; Martin 2013; McCoy 2014; Burdett 2014). In *Lithothaminon glaciale*, a high latitude species, there is a response of Mg-O bond strength to marine carbonate chemistry within the algal high Mg skeleton driven by the rate of pH reduction (Kamenos 2013). The dependency of crystal lattice integrity on carbonate chemistry variability indicates the potential for Mg-O bond strength to act as a carbonate chemistry proxy.

5.2 Hypotheses

In this study I investigate the application of coralline algal skeletal Mg-O bond strength as a carbonate chemistry proxy. It is expected that:

1. Moderate and high OA treatments (high pCO_2 / low pH) will alter the structural integrity of coralline algae, altering their Mg-O bond strength
2. A relationship will exist between Mg-O bond strength and carbonate chemistry parameters
3. Mg-O bond strength may be influenced by seasonal variability

5.3 Results

5.3.1 Influence of pCO2 on Structural Properties

Bond strengths were higher in all treatments following incubation (Figure 5.1).

Significant differences in bond strengths only occurred in the pCO_2 enrichment treatments (380: $F=0.68$, $df = 1$, $p = 0.420$, 750: $F = 9.65$, $df = 1$, $p < 0.01$, 1000: $F = 18.65$, $df = 1$, $p < 0.01$) (Supplementary Material, Table Series 6).

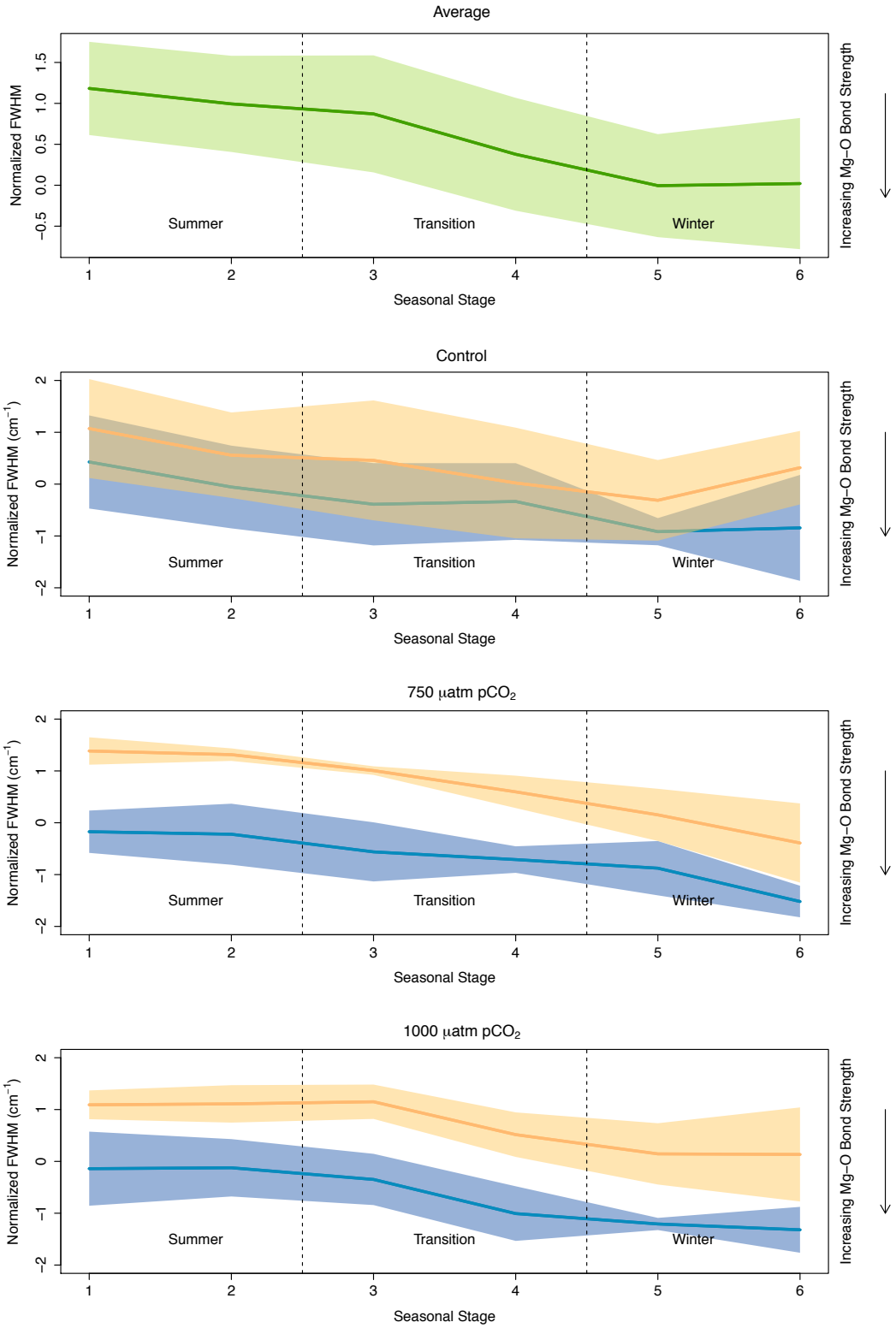


Figure 5.1: Average pre-treatment seasonality in full width half maximum (FWHM) indicative of Mg ion positional disorder / bond strength for all samples. Average normalized FWHM for control treatment, 750µatm pCO_2 treatment and 1000µatm pCO_2 treatment. Orange lines represent growth pre-treatment, blue lines represents growth during treatment and shaded area indicates standard deviation. Seasonal stage 1-2 represents summer, 5-6 winter and 3-4 transition.

5.3.2 Relationship Between pCO2 and Mg-O Bond Strength

There is a negative relationship between pCO_2 and FWHM when allowing for seasonally driven Mg concentrations present within the algae (Table 1, Figure 5.2).

Relationship	**R^2**	**Intercept SE**	**Gradient SE**	**F**	***p***	**pCO_2 FWHM $^{-1}$**
FWHM = -835 – 0.000521*pCO_2 + 0.7678*Mg	0.32	140.30	0.00025	20.2	>0.001	87.9 µatm

Table 5.1: pCO_2-FWHM relationship with associated R2 value, ± standard errors on intercept and gradient, F and p statistics. pCO_2 in µatm. FWHM = normalized full width half maximum (indicative of positional disorder / Mg-O bond strength). Mg = frequency of 1089 cm^{-1} raman shift peak (positively correlated with Mg concentration).

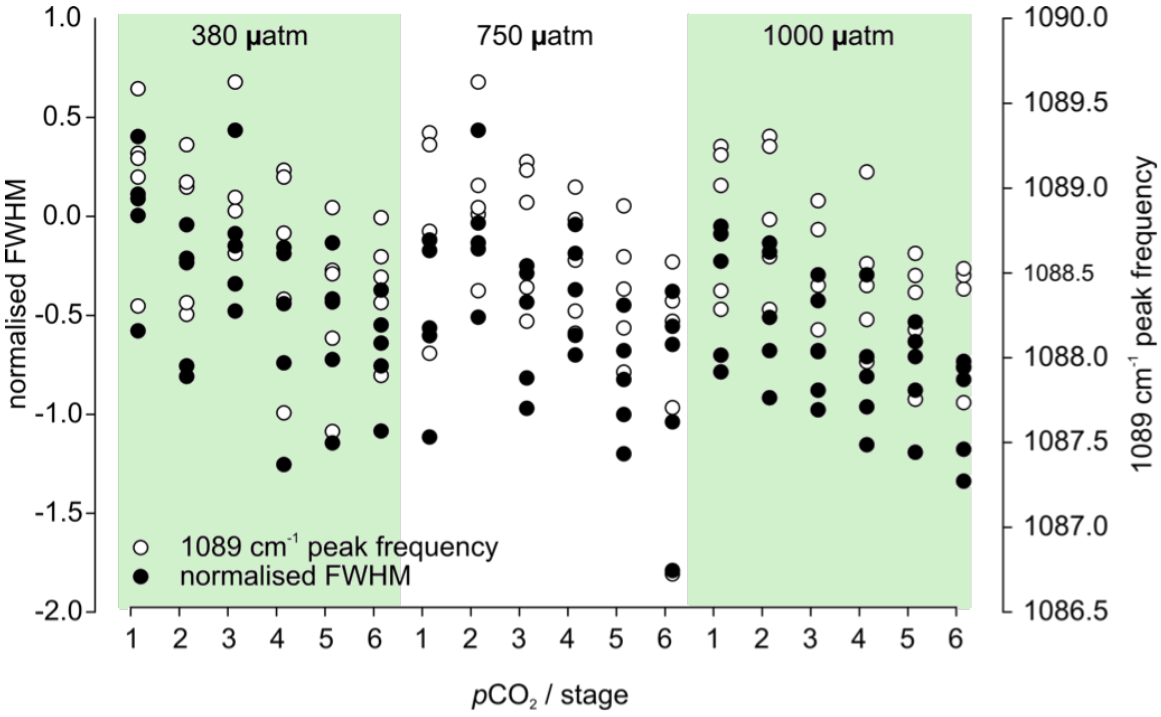


Figure 5.2: Normalized FWHM (allowing for stage-based Mg influence) in each sample for each temporal stage at 380, 750 and 1000 µatm pCO_2 (shaded area). FWHM is not as adjusted from Mg concentration.

5.4 Discussion

Mg-O bond strength within the high Mg calcite skeleton of the coralline alga Lithothamnion *glaciale* increases with increasing marine pCO_2 concentrations; this forms the basis for their utility as a pCO_2 proxy. While pCO_2 and other carbonate chemistry parameters can co-vary, pCO_2 was used to control the experimental carbonate chemistry and was the most stable carbonate parameter so was it was deemed to be the forcing factor driving changes in Mg-O bond strength. The process by which pCO_2 acts on coralline algae was likely via HCO_3- in the carbonate chemistry system as HCO_3- is a key carbon source used by red algae during photosynthesis and calcification (Koch 2013; Johnson 2014). The quantity of HCO_3- available within the ambient seawater influences the intercellular saturation and presumably the capacity to grow (through deposition of calcified material). Additionally, the energy required to undergo calcification and growth is provided by photosynthesis. The intrinsic linkage between calcification, growth and photosynthesis is compounded by the organism's biological control over these physiological processes as a consequence of natural environmental variables (Johnson 2014; Nash 2011; Ries 2011). Each of these factors influences the ability of red coralline algae mineralogical structures to acclimatize and respond to pCO_2 treatments.

5.4.1 Natural Seasonal Variability

Coralline algae show natural seasonal variability in Mg-O bond strength, with lower strengths present during summer months across treatments (Figure 5.1, Figure 5.3). Structurally, bond strength increases as a consequence of increased position disorder when CO_3 ions move out of the a-axis into the c-axis (Bischoff 1985). Thus during summer, the skeleton exhibits the least positional disorder in

the calcite lattice; this may be due to optimal summer light and temperature growing conditions required for photosynthesis (Ries 2009), causing better-controlled skeletal deposition.

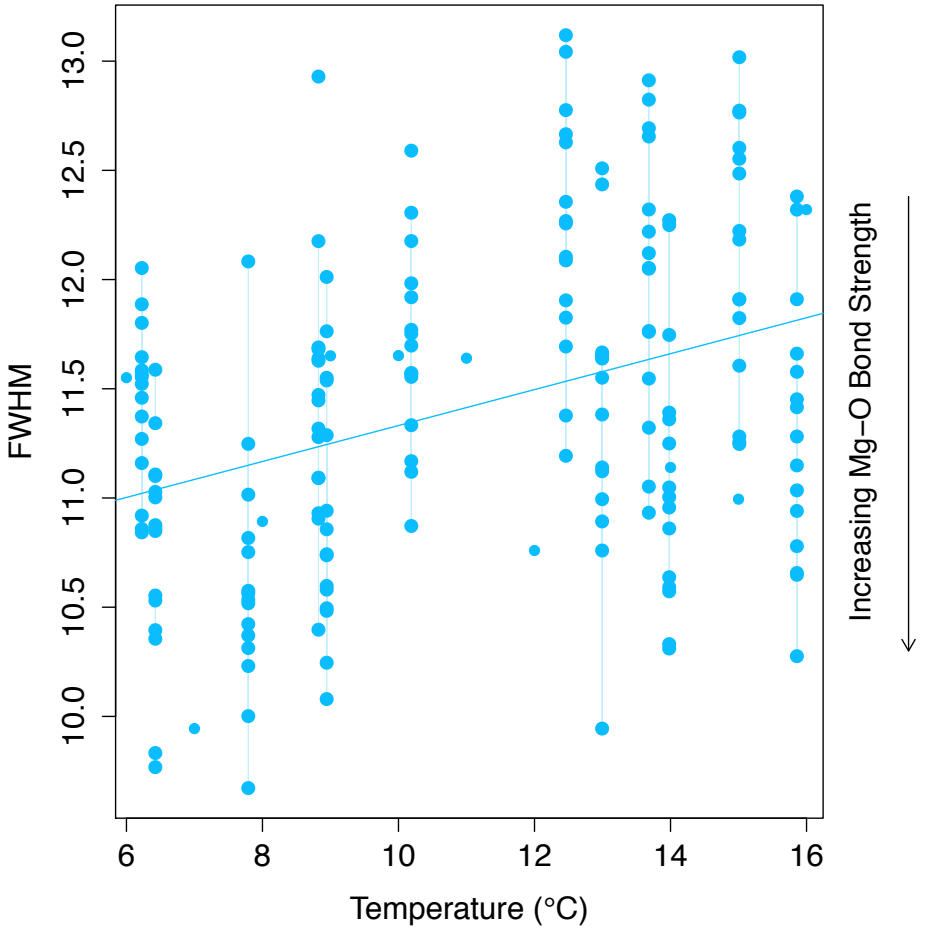


Figure 5.3: Linear relationship between FWHM and temperature.

During winter, increased positional disorder may be a consequence of sub-optimal growth conditions. This seasonal pattern in Mg-O bond strength is complemented by the seasonal variability in magnesium concentration (Chapter 4). The higher magnesium concentration in the summer is conducive of an enhanced ability to

uptake magnesium over calcium in higher temperatures (Katz 1973). During the summer months, where magnesium is abundant within the carbonate skeleton, order and stability is superior to the relative disorder of the winter months (Chave 1954; Katz 1973; Lea 1999). This is a direct consequence of the sub-optimal growing conditions occurring in high latitude winter, when photosynthesis is in a decelerated state (Martin 2009). This seasonal divergence is pronounced in the mid-latitude *L. glaciale* utilized in this research and would be further pronounced in higher latitude samples (Martin 2009).

The predictable seasonal Mg patterns (Chapter 4) in positional disorder and Mg-O bond strengths underline the importance of either comparing high Mg calcite that was deposited at the same time of year for different pCO_2 treatments or allowing for the concentration of Mg present. If Mg-O bond strengths were compared at different seasons, they would represent a highly divergent ambient environment in terms of both carbonate chemistry and physical processes. As stated, such deviations decidedly control the response of *L. glaciale* structural mineralogy.

Differences in Mg-O bond strengths observed between growth pre-collection and during pCO_2 treatments can be attributed to the impact of the pCO_2 concentrations on calcite deposition in the pCO_2 enriched treatments, but not the control, where no significant differences were observed. The latter is important as it excludes handling impacts as a driving variable in observed changes of the pCO_2 enriched treatments.

5.4.2 The Influence of pCO_2 on Coralline Algal Structure

At both 750 and 1000 µatm pCO_2 there was an increase in high Mg calcite positional disorder / Mg-O bond strength in *L. glaciale* (Figure 5.1 & 5.2). We attribute this to sub-optimal growth conditions at high pCO_2 concentrations. Although higher growth would be expected at high pCO_2 in photosynthesisers, red coralline algae dissolve at night under high pCO_2 and then hypercalcify during the day (Kamenos et al., 2013). Rapid hypercalcification is likely to lead to poor control over skeletal deposition resulting in greater positional disorder and thus Mg-O bond strength. Additionally, photosynthesis is only augmented under enhanced pCO_2 to a certain threshold, driven by the balance between the utilization for primary production and degradation of CO_3^{2-} molecules (Ries 2011). However, it has been suggested that coralline algae may broaden this threshold by intercellular utilization of bicarbonate ions for local conversion into carbon dioxide (Johnson 2014). When the balance is shifted to no longer favour photosynthesis, coralline algae may undergo alterations in physiological processes including growth and calcification (Johnson 2014; Ries 2011).

A single significant negative relationship between FWHM and pCO_2 exits across calcite deposited in all seasons when allowing for Mg concentrations (Table 5.1). Adjusting for Mg concentration within the calcite skeleton is preferential to generation of season-specific relationships as this: 1) accounts for differences in inter annual temperature causing a temporal offset in reconstructed pCO_2 and 2) minimizes variability introduced by the within-growth band location of each analysis.

5.4.3 Temporal Resolution

Six analytical sites per growth band were assessed in *L. glaciale* fixing temporal sampling at two-monthly resolution. However, fortnightly resolution temperature reconstructions have been achieved from *L. glaciale* when using Mg palaeothermometry (Kamenos 2008). Thus, with suitable instrumental parameters, Raman laser spot size can be <10 µm suggestions similar fortnightly resolution pCO_2 reconstructions may also be possible for *L. glaciale*.

5.4.4 Proxy Temporal Stability

Despite their high Mg calcite skeleton, it would appear that coralline algae may be able to lower their Mg content as a protective measure reducing skeletal reactivity at higher pCO_2s (Figure 4.1). This could be due to; 1) preferential Mg leaching or 2) reduction of skeletally-incorporated Mg by the algae themselves thereby reducing solubility and vulnerability to acidified conditions (Kamenos et al., 2013; Ries et al., 2009). In addition, OA induced Mg-O bond strength changes may be enhanced with secondary environmental stressors, including temperature (Chapter 3). Specifically, historic changes in temperature will also have changed skeletal Mg concentrations as well as Mg-O bond strength, shown here at a seasonal scale (Figure 5.1), this further reducing skeletal reactivity to reduced pH. This stresses the importance of including Mg concentrations in any carbonate system reconstructions using Mg-O bond strength.

5.5 Conclusions

Red coralline algae show significant potential to act as pCO_2 proxies via use of their skeletal bonding strength. High latitude species, including, *L. glaciale*, are of particular importance in detecting historic changes in pCO_2 enrichment as these species may experience the first pCO_2 enrichment via OA due to high latitude

shoaling of the $\Omega_{Calcite}$ depth (Feely 2004). Moreover, there is a paucity of potential high-latitude shallow-water OA proxies in comparison to developments being made with equatorial and deep-water proxies including corals and foraminifera. I determined relationships between pCO_2 and coralline algal skeletal Mg-O bond strength, a key step in understanding the capacity of molecular bonding strength to record pCO_2. This provides a tool to reconstruct palaeo- pCO_2, not only a key carbonate chemistry parameter but also a critical parameter for understanding historic atmospheric pCO_2 concentrations.

CHAPTER 5 SUMMARY

- The reliance of red coralline algae (magnesium calcite) structure on carbonate chemistry parameters provides the basis to their use as a oceanic pCO_2 proxy
- The bond strength of magnesium and oxygen within high magnesium calcium carbonate varies seasonally and is positively correlated to positional disorder of CO_3 within the crystal lattice; higher order is achieved during summer when growing conditions are optimal
- Mg-O bond strength increases linearly with increasing pCO_2 with a dependence on relative magnesium concentration with high significance; this linear relationship provides a theoretical basis which can be calibrated across latitudes to develop a new high-resolution proxy

SUMMARY OF SIGNIFICANCE & OBJECTIVES

The influence of anthropogenic climate change on the marine biosphere via ocean acidification and thermal stress is well documented. The purpose of this thesis was to supplement the current scientific knowledge, which necessitates greater spatial, temporal and organismal resolution (IPCC 2013, AMAP 2013). This was undertaken by looking both into the future and the past, through the analysis of a high latitude, calcifying marine organism red coralline algae (RCA).

The effects of ocean acidification and enhanced temperature on marine organisms are two fold, by stressing both photosynthesis and calcification two processes required for the growth and propagation of RCA (Bosence 1983, Birkett 2003, Luning 1990). Such stress may also have radiating effects on other biological functions.

Marine mesocosms were used to replicate a naturally dynamic environment under various controlled temperature and pCO_2 regimes, representative of current and future conditions, in order to understand:

1. The individual and collective influence of temperature and CO_2 stress on RCA linear extension (growth);
2. The effect of temperature and seasonality on relative magnesium concentrations within RCA's high magnesium calcium carbonate structure;
3. Testing the relationship between pCO_2 and magnesium-oxygen bond strength and its use as a palaeoclimate proxy, knowing magnesium seasonality

Many studies that focus on the integrated stress of temperature and CO_2 maintain mesocosms for short period of time (Martin 2009, Marin 2012, Jokiel 2008, Ragazolla 2012, Budenbender 2011), not permitting time for organism acclimatization or consideration of seasonal variability. The methodology in this thesis reduced the uncertainty associated with a short incubation time by allowing samples to grow for 24 months before analysis across seasonal transitions.

Red coralline algae was chosen as it has a high capacity as a marine palaeoclimate proxy, by means of its distribution from the equator to the poles, high biweekly resolution (from seasonal (stable) growth bands), availability throughout the fossil record and its ability to record environmental conditions with trivial vital effects (Wilson 2004, Nelson 2009, Adey 1973, Nelson 2009, Barbera 2003, Kamenos 2004, Bradley 1999, Halfar 2000).

The study of environmental stress in red coralline algae from the westcoast of Scotland is important since high latitude ocean are expected to experience the first signs of ocean acidification because cold water more readily uptakes atmospheric CO_2 (IPCC 2013). Moreover, acute stress on maerl beds may have radiating effects on organisms that are reliant on the microhabitats within the complex calcium carbonate framework (Kamenos 2003, Freiwald 1994, Adey 1998, Bosence 1983).

Research Outcomes

The preceding research highlights the complex influences of climate change indices on the growth, composition and structure of the high latitude red coralline algae species, Lithothamnion *glaciale*. The following points summarize the principal resulting concepts discovered:

1. Temperature is the primary driver of growth (linear extension) dynamics in L. *glaciale*. An increase of 2°C, controlled in a seasonal pattern, proved to have a significantly negative effect on growth. The consequential growth reduction in high temperature samples is likely due to the limitation of carbon metabolism / carbon dioxide assimilation during the photosynthesis process during the summer. Higher temperatures during the summer are expected to have stressed the L. *glaciale*, while growth may have been stimulated during the winter.
2. The quantity of growth was not significantly affected by an increase in pCO_2 to moderate (750µatm) or high (1000µatm) ocean acidification. The lack of response may be a result of the L. *glaciale*'s ability to acclimatize to enhanced pCO_2 levels, possibly by utilizing other carbon sources (bicarbonate). Additionally, though the *quantity* of growth may not have been affected, the *quality* may have been reduced through a decline in calcification.
3. The multiple factor analysis of combined temperature and pCO_2 increases yielded insignificant results. Moderate and high ocean acidification treatments in combination with increased temperature generally enhanced total growth in comparison to high temperature alone. However, these results were highly variable.
4. The relative magnesium concentrations within the calcite structure were found to be significantly correlated to ambient ocean temperature for both *in-situ* and incubation measurements. Magnesium was more readily incorporated into the calcite structure in higher temperatures.

5. The quantity of magnesium incorporated was generally lower in incubation treatments (especially during the winter), likely due to acclimatization. A reasonable reduction was only found in the high ocean acidification treatments, mirrored in the temperature-Mg relationship by a reduction in ANCOVA significance. This result may be due to a reduced ability for the L. *glaciale* to calcify efficiently. Nonetheless, this consequence is not strong enough to reduce the ability to utilize L. *glaciale* as a paleothermometer.
6. The bond strength between magnesium and oxygen within the high magnesium calcite structure is generally weaker during the summer. During the summer, when growth conditions are optimal, the L. *glaciale* structure can control the positional disorder with occurs as a consequence of magnesium being incorporated into the structure (tilting the CO_3 out of plane). During the winter, this growth is more erratic, due to a reduction in temperature and light, resulting in less control over structural properties.
7. Mg-O bond strengths (and therefore positional disorder) increase proportionally with pCO_2, which is the basis for the paleoproxy. This proxy is influenced by the seasonality in magnesium concentration, and therefore it must be considered when calculating the relationship.

The cumulative results provide important insight into the future of maerl beds and their associated ecosystems: thermal stress reduces the ability of *L. glaciale* to grow and surpasses the effects of moderate to high ocean acidification. Though growth was not significantly influenced by ocean acidification, the internal structure of *L. glaciale* was altered by means of an increased internal disorder of magnesium-oxygen bonds. This demonstrates that though linear extension was not reduced in acidic conditions, the quality of growth was affected, which is the basis of the aforementioned novel pCO_2 proxy.

Future Considerations

The research outcomes represent a first step in understanding the complex dynamics of high latitude red coralline algae structure and growth under the influence thermal and chemical drivers. Building upon the knowledge gained from the current study, the following should be considered for future research:

1. Investigating the effects of *both* growth and calcification at additional temperature steps. Moreover, the temperatures could be increased incrementally to allow for the examination of acclimatization. Sampling during such increments at different seasons would permit the analysis of the extreme seasonality experienced by high latitude red coralline algae.
2. The relative magnesium concentration analyzed could be investigating using absolute measurements from electron microprobe analysis similar to Kamenos 2008. This addition to the incubation process would provide quantifiable conclusions to how pCO_2 may influence the use of red coralline algae as a paleothermometer.
3. The Mg-O bond strength paleoproxy should be calibrated utilizing samples at numerous pCO_2 steps, rather than just 380µatm, 750µatm and 1000µatm. Additionally, samples from tropical, temperature and arctic environments should be compared.

Conclusions

The impacts of climate change on the structure, growth and propagation of red coralline algae (RCA) has radiating consequences throughout the marine biosphere. Articulated RCA provide a basis for diverse ecosystems, with high abundance from the tropics to the poles in coral reefs and maerl beds alike. The slow, incremental growth of well-defined seasonal bands provides a foundation for investigations in the influence of physical and chemical parameters through time.

Our research has helped circumscribe the dynamics and influences of selected environmental parameters on *L. glaciale*. Temperature was found to have a significant negative effect on growth, deviating from the current knowledge (Ragazolla 2012, Bunderbender 2011), likely as a result of the acclimatization period and correlation to natural seasonal variability. This result demonstrates the importance of paralleling natural conditions as close as possible for as long as possible to gain accurate results.

Contemporaneous climate change, driven by anthropogenic influences, has made the search for such proxies essential to understanding how such perturbations in the earth system will influence the biosphere, by using analogues from the past. The intricacies of ocean change are particularly important as the atmospheric chemical balance depends on the uptake of CO_2 by the ocean sink. The thresholds and feedbacks associated with of oceanic uptake may result in adaptive, non-linear or chaotic consequences. Reaching the boundary of the ocean sink may lead to impulsive change or a downward trend in the tendency for the sink to uptake CO_2. For this reason, the development of a paleoproxy, which describes the ocean carbon cycle across latitudes, in important biological zones will assist in understanding such future system variability. The novel pCO_2 proxy tested within this thesis provides an alternative to the relatively unreliable and very complex pH proxy using boron isotopes.

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SUPPLEMENTARY MATERIAL

Table 1: Carbonate Chemistry Parameters

Season	Treatment	Temperature (°C)	Salinity	pH (T)	Alkalinity (µmol kg^{-1})	DIC (µmol kg^{-1})	HCO_3 (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	pCO_2	Ω_{cal}	Ω_{arag}
Summer	380	16.1 ±0.2	34.9 ±0.8	8.0 ±0.1	1195.1 ±108.5	1108.6 ±96.1	1048.4 ±44.2	48.6 ±8.5	383.1 ±25.1	1.2 ±0.2	0.7 ±0.1
	750	15.8 ±0.4	33.7 ±1.8	7.8 ±0.1	1532.9 ±198.7	1480.7 ±182.2	1413.4 ±170.6	42.8 ±11.4	747.6 ±13.5	1.1 ±0.3	0.64 ±0.2
	1000	15.7 ±0.2	34.5 ±1.9	7.6 ±0.1	1533.5 ±219.7	1518.1 ±200.3	1449.6 ±193.5	30.0 ±10.9	1138.7±119.8	0.7 ±0.2	0.4 ±0.1
Transition	380	13.2 ±0.9	35.5 ±1.7	8.1 ±0.1	1432.2 ±365.3	1322.5 ±327.0	1243.4 ±298.6	66.2 ±28.3	364.4 ±3.6	1.6 ±0.7	1.0 ±0.4
	750	12.9 ±0.5	33.5 ±0.5	7.8 ±0.1	1627.6 ±279.1	1580.8 ±261.5	1509.9 ±247.9	43.0 ±13.3	758.1 ±0.7	1.0 ±03	0.7 ±0.2
	1000	12.9 ±1.3	33.8 ±1.4	7.8 ±0.1	2086.1 ±544.5	2041.4 ±516.2	1949.9 ±490.2	53.1 ±24.2	1041.9±21.3	1.3 ±0.6	0.8 ±0.4
Winter	380	7.4 ±0.7	35.2 ±2.0	8.1 ±0.1	1910.1 ±400.8	1777.3 ±346.9	1668.7 ±306.8	91.8 ±40.8	374.9 ±17.8	2.2 ±1.0	1.4 ±0.6
	750	7.2 ±0.6	33.9 ±1.5	7.8 ±0.0	1950.5 ±196.5	1904.7 ±186.2	1820.1 ±176.5	49.7 ±8.7	758.1 ±58.6	1.2 ±0.2	0.8 ±0.1
	1000	7.1 ±0.6	35.2 ±2.5	7.3 ±0.0	2363.3 ±346.2	2339.2 ±334.1	2235.7 ±319.0	50.6 ±12.5	1142.8±69.4	1.2 ±0.3	0.8 ±0.2

Table 2: Linear Extension measurements

Identifiers		Conditions		Measurements (um)					Mean	Standard Deviation
Code	Sample #	Temperature	pCO2	1	2	3	4	5		
H-380-1	1	H	380	0	0	0	0	0	0	0
H-750-1	2	H	750	38	41.6	29	32.8	35.6	35.4	4.820788317
H-380-2	3	H	380	0	0	0	0	0	0	0
H-750-2	4	H	750	10.4	0	0	0	5.4	3.16	4.674184421
H-380-3	5	H	380	0	0	0	0	0	0	0
H-750-3	6	H	750	102.4	115.6	198.4	222.4	230.8	173.92	60.62435154
H-380-4	7	H	380	0	0	0	0	0	0	0
H-750-4	8	H	750	0	0	2.26	0	2.64	0.98	1.348628933
H-380-5	9	H	380	56	18.4	63.68	67.68	63.2	53.792	20.22763259
H-750-H	10	H	750	46.8	36.8	36.4	33.6	46.4	40	6.1514226
H-380-6	11	H	380	0	0	0.8	0	1.2	0.4	0.565685425
H-750-6	12	H	750	102.8	104	59.2	107.2	81.6	90.96	20.43448066
H-380-7	13	H	380	0	0	0	0	0	0	0

H-750-7	14	H	750	63.2	45.92	34.04	50.88	44.36	47.68	10.62167595
H-380-8	15	H	380	33.4	35.08	21.12	24	24.64	27.648	6.19053471
H-380-9	16	H	380	0	0	0	0	0	0	0
H-1000-1	17	H	1000	0	0	0	0	0	0	0
H-1000-2	18	H	1000	175.46	184.8	148.06	147.86	183.68	167.972	18.62104508
H-1000-3	19	H	1000	40.88	7.28	54.02	42.88	48.44	38.7	18.29625098
H-1000-4	20	H	1000	0	1.6	0	2.08	1.12	0.96	0.93978721
H-1000-5	21	H	1000	31.96	49.02	39.06	48.72	37.3	41.212	7.464403526
H-1000-6	22	H	1000	0	0	0	0	0	0	0
H-1000-7	23	H	1000	54.48	60.36	45.8	65.64	47.88	54.832	8.327707968
L-380-1	24	L	380	58.7	73.24	88.06	87.02	62.24	73.852	13.60201897
L-750-1	25	L	750	180.16	206.44	194.8	196.68	215.92	198.8	13.4108911
L-380-2	26	L	380	4.56	0	0	2.24	7.76	2.912	3.302653479
L-750-2	27	L	750	237.16	210.52	249.64	206.44	225.6	225.872	18.06721672
L-380-3	28	L	380	303.64	304.68	287.56	255.64	207.52	271.808	41.03754769
L-750-3	29	L	750	104.28	115.58	88.02	99.44	92.12	99.888	10.80417142
L-380-4	30	L	380	111.7	114.58	90.36	118.6	60.2	99.088	24.33226911
L-750-4	31	L	750	39.36	46.54	49.58	46.02	41.1	44.52	4.190942615
L-380-5	32	L	380	564.92	517.56	468.12	421.12	314.2	457.184	96.32878531
L-750-5	33	L	750	0	0	0	0	0	0	0
L-380-6	34	L	380	250.1	283.64	302.52	309	266.36	282.324	24.56855063
L-750-6	35	L	750	257.76	271.12	290.16	275.08	245.52	267.928	17.03752095
L-380-7	36	L	380	163.54	151.76	119.68	129.1	147.9	142.396	17.73307306
L-750-7	37	L	750	130.46	136.16	137.92	91.88	123.36	123.956	18.81128597
L-380-8	38	L	380	368.16	361.36	339.8	336.92	359.2	353.088	13.88274901
L-750-8	39	L	750	0	0	2.26	3.87	0	1.226	1.772647737
L-380-9	40	L	380	176.32	172.04	167.44	165.44	186.96	173.64	8.556354364
L-750-9	41	L	750	0	0	0	0	0	0	0
L-750-10	42	L	750	62.38	52.04	44.36	62.64	28.44	49.972	14.26389568
L-380-10	43	L	380	0	0	0	4.8	2.6	1.48	2.170714168
L-380-11	44	L	380	33.64	40.04	44.12	39.32	32.2	37.864	4.89682346
L-1000-1	45	L	1000	154.84	249.28	306.44	300.04	287.52	259.624	62.62999026
L-1000-2	46	L	1000	39.14	39.62	39.14	36.06	32.06	37.204	3.205570152
L-1000-3	47	L	1000	242.64	287.4	302.16	260.32	294.2	277.344	24.97631838
L-1000-4	48	L	1000	286.3	268.88	282.08	276.76	273.44	277.492	6.887519147
L-1000-5	49	L	1000	31.94	31.52	30.1	30.92	32.06	31.308	0.809271277
L-1000-6	50	L	1000	57.9	48.1	52.1	54.08	57.14	53.864	3.981316365
L-1000-7	51	L	1000	89.46	102.5	100.7	101.52	99.18	98.672	5.291419469
L-1000-8	52	L	1000	181.16	196.88	202.2	224.76	207.04	202.408	15.83624703
L-1000-9	53	L	1000	154.1	149.78	130.1	118.92	134.08	137.396	14.47391723

Table 3: Linear Extension measurements summary

Group	n	Average	Standard Deviation	No Growth Individuals
High 380	9	9.093333333	2.998205858	78%
High 750	7	56.01428571	15.52507606	29%
High 1000	7	43.38228571	7.664170681	42%
Low 380	11	172.3305455	22.76468539	18%
Low 750	10	101.2162	9.835857219	30%
Low 1000	9	152.8124444	15.3435077	0%

Table Series 4: ANCOVA results for growth (linear extension)

a

ANCOVA (Type 3 SS)				
Response: Growth				
	SS	**Df**	**F**	**p**
Treatment	4877	1	0.5025	0.481761
Temperature	56445	1	5.8163	**0.01967**
Treatment: Temperature	11162	1	1.1502	0.288771

b

Linear Model: accounting for lack of interaction				
	Estimate	**Standard Error**	**t**	**p**
Treatment	3.81E-04	5.26E-02	0.007	0.99424
Temperature	-5.45E+01	1.37E+01	-3.984	**0.00022**
Multiple R-squared: 0.241 **F-statistic:** 7.94 **p-value:** 0.001013				

c

ANCOVA: Low Temperature (Type 3 SS)				
Response: Growth				
	SS	**Df**	**F**	**p**
Treatment	4877	1	0.3203	0.57596

d

Linear Model: Low Temperature				
	Estimate	**Standard Error**	**t**	**p**
Treatment	-0.04991	0.08819	-0.566	0.576
Multiple R-squared: 0.01131 **F-statistic:** 0.3203 **p-value:** 0.576				

e

ANCOVA: High Temperature (Type 3 SS)				
Response: Growth				
	SS	**Df**	**F**	**p**
Treatment	6286	1	2.6851	0.1162

f

Linear Model: High Temperature				
	Estimate	**Standard Error**	**t**	**p**
Treatment	0.06345	0.03872	1.639	0.116
Multiple R-squared: 0.1134 **F-statistic:** 2.685 **p-value:** 0.1162				

g

ANCOVA: Excepting Temperature (Type 3 SS)				
Response: Growth				
	SS	**Df**	**F**	**p**
Treatment	46	1	0.0037	0.95198

h

Linear Model: Excepting Temperature				
	Estimate	**Standard Error**	**t**	**p**
Treatment	0.06345	0.03872	1.639	0.116
Multiple R-squared: 7.181e-05 **F-statistic:** 0.003663 **p-value:** 0.952				

Table Series 5: ANCOVA results for magnesium concentration

a

ANCOVA Control Treatment Response: Mg Concentration				
	SS	**Df**	**F**	**p**
Temperature	0.7064	1	49.241	**0.0197**
Stage	0.2211	1	15.415	**0.0598**
Temperature : Stage	0.0329	1	2.296	0.269

b

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	0.7064	1	34.39	**0.00989**
Stage	0.2211	1	10.77	**0.04639**

c

ANCOVA 750uatm Treatment Response: Mg Concentration				
	SS	**Df**	**F**	**p**
Temperature	0.17287	1	47.75	**0.0203**
Stage	0.03821	1	10.553	**0.0831**
Temperature : Stage	0.00622	1	1.719	0.3202

d

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	0.17287	1	38.521	**0.00843**
Stage	0.03821	1	8.513	**0.06161**

e

ANCOVA 1000uatm Treatment Response: Mg Concentration				
	SS	**Df**	**F**	**p**
Temperature	0.28857	1	42.933	**0.0225**
Stage	0.03457	1	5.143	0.1515
Temperature : Stage	0.0004	1	0.059	0.8305

f

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	0.28857	1	62.55	**0.00421**
Stage	0.03457	1	7.493	**0.0715**

g

ANCOVA *In-situ* Response: Mg Concentration				
	SS	**Df**	**F**	**p**
Temperature	0.23089	1	16.609	**0.00114**
Stage	0.06929	1	4.984	**0.04243**
Temperature : Stage	0.00212	1	0.153	0.70199

h

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	0.23089	1	17.603	**0.00078**
Stage	0.06929	1	5.282	**0.03634**

i

Treatment Linear Mode: Temperature -- Stage				
	SS	**Df**	**F**	**p**
Stage	50.32	1	9.321	**0.0379**

j

In-situ Linear Mode: Temperature -- Stage				
	SS	**Df**	**F**	**p**
Stage	98.91	1	25.59	**0.000116**

Table Series 6: ANCOVA results for FWHM (bond strength)

a

ANCOVA Control Treatment Response: Post-FWHM				
	SS	**Df**	**F**	**p**
Pre-FWHM	0.292	1	0.68	0.42
Stage	2.425	1	1.13	0.38
Pre-FWHM : Stage	0.175	1	0.082	0.994

b

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	0.292	1	0.85	0.366
Stage	2.425	1	1.411	0.257

c

ANCOVA 750uatm Treatment Response: Post-FWHM				
	SS	**Df**	**F**	**p**
Pre-FWHM	3.138	1	9.652	**0.00609**
Stage	0.197	1	0.121	0.98591
Pre-FWHM : Stage	0.138	1	0.085	0.99378

d

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	3.138	1	12.05	**0.00207**
Stage	0.197	1	0.151	0.97759

e

ANCOVA 1000uatm Treatment Response: Post-FWHM				
	SS	**Df**	**F**	**p**
Pre-FWHM	6.755	1	18.651	**0.000414**
Stage	0.65	1	0.359	0.869711
Pre-FWHM : Stage	1.21	1	0.668	0.652704

f

Linear Model: accounting for lack of interaction				
	SS	**Df**	**F**	**p**
Temperature	6.755	1	20.102	**0.000169**
Stage	0.65	1	0.387	0.852575